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THE PHYSIOLOGY OF THE PARATHYROID GLANDS

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The recent extraction of an active principle from the parathyroid glands, capable of affording a successful substitution therapy, and the development of various methods by means of which experimental animals may be made to survive the removal of the parathyroids without specific therapy, have greatly stimulated and facilitated the study of the physiology of these glands. It is not possible to make an adequate survey of the older literature in this field within the scope of a brief review. An attempt is made to analyze the present state of our knowledge and reference will be made to earlier work only as it bears directly on the current problem. The reviews of Carlson (1924), Biedl (1922), Simpson (1924), Boothby (1921), Paton and Findlay (1916), MacCallum (1924), Jacobson (1924) and others deal quite adequately with this literature.

ANATOMY OF THE PARATHYROID GLANDS. The parathyroid glands appear first as definite structures in the amphibia, and are present and, so far as is known, exert an analogous function in reptiles, birds and mammals. They are developed from dorso-cranial thickenings of the epithelium of the third and fourth branchial pouches in the embryo in mammals, and accordingly are often intimately associated anatomically with the thymus, which arises from ventral outgrowths of these same structures. In their further development the parathyroids become separated from the primitive gullet and in many animals are more or less completely enclosed within the lateral lobes of the thyroid, which develop from the epithelium of the fifth branchial pouch. Commonly there are four glands, two closely associated with each thyroid lobe and a varying number of islands or nodules of accessory parathyroid tissue in the connective tissue surrounding the thyroid (Pepere, 1908), in the thymus (Hammar, 1910; Pappenheimer, 1910; Nicholas and

Swingle, 1925), or in the connective tissue in the anterior mediastinum, along the trachea, arch of the aorta, or pulmonary artery (Biedl, 1922). In mammals the four glands weigh less than half a gram (Welsh, 1898). They are made up of strands of epithelial cells, supported by a reticulum of endothelial cells of varying size and shape (Williamson and Pearse, 1926) and enclosed by a connective tissue capsule. Often there is no definite columnar arrangement, the cells appearing in masses or clumps, but sometimes there is follicle formation containing colloid, the significance of which is unknown. It has however no relation to the thyroid colloid, being entirely uninfluenced by factors which alter the latter. Furthermore, the parathyroids contain no more iodine than is found in organs such as the ovary, pituitary or thymus (Chenu and Morel, 1904; Estes and Cecil, 1907; Hjort, Gruhzit and Flieger, 1925). The parenchyma consists of fairly large polygonal cells, with deeply staining nuclei and poorly staining cytoplasm, the chief cells, and a smaller number of slightly smaller cells with granular deeply staining cytoplasm and small central nuclei, the chromophile cells. Tomaszewski (1918) found a proportionate increase in the chromophile cells in cases where he found abnormal deposits of calcium as in the arterial walls, rib cartilages, lymph glands, tuberculous nodules, and which he ascribed to hyperactivity of the parathyroid glands. A similar abnormal deposition of calcium has been observed following the excessive administration of active parathyroid extracts to dogs (Hueper, 1927). A marked hypertrophy of the parathyroids together with a proportionate increase in the chromophile cells and increased vascularity, is a constant finding in pregnancy (Seitz, 1920; Erdheim, 1925). The blood supply to the parathyroids is by way of the superior and inferior thyroid arteries and is very abundant. Nerves to the parathyroids accompanying the blood vessels have been described, some of which terminate in the vessel walls but many of which penetrate between the epithelial cells forming nodular endings (Rhinehardt, 1912). The function of these nerves is unknown. Transplantation experiments have demonstrated, however, that the parathyroids can function adequately in the absence of all nervous connections (Borcher, 1919).

ACUTE EFFECTS OF EXTIRPATION OF THE PARATHYROID GLANDS. *Parathyroid tetany.* Much of the knowledge of the function of the parathyroid glands has been derived from studies of the clinical symptoms and the metabolic disturbance which have been found to take place upon their partial or complete extirpation in man and in experimental animals. It is probable that the metabolic disturbance is essen-

tially the same in all species and that the variations in the symptom complex observed in different animals are dependent upon other and minor factors.

When the parathyroid glands of the dog are completely removed at one operation, there results in over 90 per cent of cases a characteristic symptom complex, parathyroid tetany or tetania parathyropriva, which has its counterpart in most other species. The recovery from the operation is complete and for a varying period of from 12 hours to 3 or 4 or rarely 5 to 6 days the animal may appear entirely normal. In most cases the earliest sign of any disturbance is an apparent loss of appetite, the refusal of food and water, and an increasing restlessness At this time if food be introduced into the stomach by a tube it will usually be promptly vomited. Upon examination the peripheral motor nerves will be found hyper-excitable to electrical but not to mechanical stimulation. Respiration is increased. Often the animal thrusts its hot dry nose between the steel bars of its cage or scratches it frantically. Paroxysms of sneezing are a common feature in some dogs and in these respiration may be difficult. A diarrhea, often bloody and very offensive, is a frequent symptom. Soon fine fibrillary contractions of the temporal muscles can be felt and as these become pronounced, other muscles about the head and jaws are involved, and finally the legs. These are succeeded by more coarse irregular contractions and a gradually developing spasticity. If the stomach tube is now introduced, the esophagus and in particular the cardia will be found tonically contracted. There is a slight enophthalmos, the nictitating membrane is drawn partly over the eye, and the spasm of the orbicularis oculi produces a definite trichiasis. The heart beat is slow and forcible and the temperature very high (106 to 107°F.) A profuse salivation now occurs, and this together with an increasing hyperpnea and clonic contractions of the leg muscles, initiates the spasm. The animal falls on its side, salivating and panting sterterously, its spastic legs stretched out and showing intermittent clonic contractions which become more frequent and severe until finally all the muscles contract in a tonic spasm, respiration stops, urine and feces are expelled, and death may occur. Usually however recovery, often surprisingly complete, takes place only to be succeeded by other and more severe attacks at progressively shorter intervals. Death is commonly produced by the asphyxia resulting from the long continued spastic contraction of the larynx and the muscles of respiration. A small number of animals die apparently from exhaustion between attacks.

There are many variations in this general picture in dogs and marked differences in other animals. Often cats and occasionally dogs are depressed from the start and the condition progresses through an apathy, somnolence, rapid cachexia, paresis, to death within two weeks (Carlson, 1912; Paton and Findlay, 1916). In the monkey the symptom complex is much milder, acute convulsions may not occur at all, the animal passing into a state of chronic tetany with progressive cachexia (Paton and Findlay, 1916).

NECESSITY OF THE PARATHYROID GLANDS FOR LIFE. It is probable that a sudden destruction or removal of all parathyroid tissue will bring about a disturbance in metabolism in most species which, if not controlled by special treatment, is fatal. However the results following extirpation of the glands vary widely in different animals, due primarily to the inconstancy in the number and site of supernumerary and accessory nodules of parathyroid tissue, which may remain. If the four glands associated with the thyroid are removed at a single operation a fatal insufficiency is produced in the dog in from 95 to 100 per cent of cases (Marine, 1914; Simpson, 1924); but in the cat only in from 50 to 80 per cent of cases (Harvier and Morel, 1909; Farner and Klinger, 1920; Nicholas and Swingle, 1925). Baggio (1913) found that fatal tetany occurred in only 13.33 per cent of rabbits following thyro-parathyroidectomy but that if the accessory parathyroid tissue in the thymus was also removed by a thymectomy, the percentage rose to 81.25.

An acute parathyroid insufficiency is much more liable to cause death than one of equal degree gradually produced. The piecemeal ablation of the parathyroids in the dog at several operations will often result in a chronic or latent tetany whereas the total extirpation at one sitting will usually cause death in acute tetany.

Age is likewise a significant factor. Young animals are much more susceptible to parathyroid deficiency than adults, and aged animals are most resistant (Horsley, 1886; MacCallum, Thompson, Murphy, 1907). The removal of only three parathyroids may cause fatal tetany in a young dog, whereas it is usually without such effect in an adult. Proportionately a much greater quantity of calcium lactate is required to control the tetany after thyro-parathyroidectomy in the young than in the adult dog (Dragstedt and Sudan, 1926). Aged dogs or sheep may be unaffected by thyro-parathyroidectomy (Horsley, 1886; Simpson, 1924).

Pregnancy markedly increases the severity of the tetany and shortens the life following parathyroidectomy in the dog (Carlson, 1913; Werelius,

1913) but not in the cat. Pregnant dogs may develop tetany within eight hours after thyroparathyroidectomy and death occur within twenty-four.

Animals that are fed largely or exclusively on meat develop tetany and die sooner following parathyroidectomy than those given meat-free diets or starved (Marine, 1914; Paton and Findlay, 1916; Blum, 1925).

Animals with active rickets are likewise more susceptible to parathyroid deficiency than normals (Marine, 1914; Paton and Findlay, 1916). Similarly excitable or highly irritable strains of rats were found by Hammett (1921, 1922) to be much more susceptible to parathyroid extirpation than were gentled animals.

The majority of all animals that die following parathyroid extirpation are killed by the convulsions incident to the acute tetany and only a minor number die from depression or cachexia. It is apparent that acute parathyroid tetany can be repeatedly relieved and controlled by measures such as the intravenous injection of Ringer's solution (Luckhardt and Rosenbloom, 1922) a milk and lactose diet (Dragstedt and Peacock, 1922) and others which do not involve the administration of any specific product of the parathyroids, and so far as is known the continued application of these methods will permit the dog, and possibly other animals, to survive as complete a parathyroid deficiency as can be experimentally produced. It is accordingly evident that the parathyroid glands do not perform a specific function in metabolism which is indispensable for life. It is not known whether the procedures which enable the parathyroidectomized dog to survive the acute tetany will also compensate for the more gradual chronic disturbances which result from deficiency of parathyroid function.

Nervous system after parathyroid extirpation. The most constant as well as the earliest change to be observed after parathyroid extirpation is the increased excitability of the peripheral nerves to electrical, and to a less extent to mechanical stimulation. The cathodal closing contraction may be normally elicited with a current of 1.0 to 4.0 milliamperes in the dog and 0.2 to 2.0 milliamperes in the cat whereas in tetany currents of 0.1 milliampere and less suffice. (Paton, Findlay, Watson, 1916; Jacobson, 1923.) This increased excitability may occur after section of the nerve and it persists until degeneration sets in, irrespective of whether the nerve be cut before, with, or after removal of the parathyroids (MacCallum and Vogel, 1913; Biedl, 1922). It is due to the direct effect of the blood on the peripheral nerve as demonstrated by the perfusion of the isolated leg of a normal dog

with tetany blood (MacCallum and Vogel, 1913; Jacobson, 1923) and the nerve ending seems to be the point acted upon (Paton, Findlay, Watson, 1916). There is little evidence however that this increased excitability is present in the sympathetic system. The injection of nicotine, adrenalin and pituitrin causes a comparatively greater increase in blood pressure in tetany animals than in the normal (Hoskins and Wheelon, 1914). The neuromuscular mechanisms of the digestive tract, bladder, rectum, anal sphincters, and uterus are normal or depressed during parathyroid tetany in cats and dogs and there is likewise no evidence of hyperexcitability of the cervical sympathetic nerves to the pupil or salivary glands (Carlson, 1912). In fact, the enophthalmos and prominent nictitating membrane during tetany point rather to a paresis than to a stimulation of the (cervical) sympathetic (Luckhardt, 1923). The increased irritability of the phrenic nerve causes it to be excited by the action current of the heart (Carlson and Jacobson, 1911). The previous section of the sympathetic nerves causes no alteration in the tonus of the legs in tetany cats or dogs (Tower, 1926). The reflexes in tetany cats and dogs are normal.

However there is no direct proportion between the degree of increased irritability of the motor nerves and the intensity of the convulsions. Section of the nerves to any part of the body abolishes all spasms and tremors of the muscles supplied (Paton, Findlay and Watson, 1916). Spinal transection abolishes the spasticity, tonic, and clonic convulsions of the part posterior (Mustard, 1912; Palmer, 1920; Carlson and Jacobson, 1911) but not the tremors and jerkings (Paton, Findlay, Watson, 1916; Luckhardt, Sherman and Serbin, 1920). The tremors and jerkings are accordingly due to action on the spinal cord and apparently on the efferent arc since they may develop after section of the dorsal roots (Paton, Findlay, Watson, 1916). Removal of the motor cortex does not alter the character or intensity of the tetany in dogs (Mustard, 1912) but in cats decerebration increases the severity of the tonic and clonic convulsions (Paton, Findlay and Watson, 1916). The tonic and clonic convulsions appear therefore to be due to an effect on the cerebellum or midbrain, which does not proceed pari passu with the increase in excitability of the peripheral nerves. The hyper-excitability is reduced after a convulsion (Paton, Findlay and Watson, 1916) but this is temporary and cannot account for the remarkable periodicity in the nervous manifestations in the parathyroidectomized animal.

ALIMENTARY TRACT AFTER PARATHYROID EXTIRPATION. Some of the early symptoms displayed by cats and dogs after parathyroidectomy

indicate a more or less severe gastro-intestinal disorder. Anorexia is common and vomiting not infrequent particularly if food is introduced into the stomach. A bloody offensive diarrhea is common in cats. Hemorrhages and ulcers are frequently found in the stomach and intestine at autopsy. Dogs frequently groan and it is evident that the abdomen is painful and tender in the interval between convulsions. The introduction of a stomach tube often reveals a spasm of the esophagus or cardia in mild tetany. There is no evidence of tetany or hyperexcitability of the neuromuscular mechanism of the stomach or intestines and in severe tetany any deviation from the normal is in the direction of depression or paralysis (Carlson, 1912). The secretion of gastrc juice is depressed (Keeton, 1914) but if the tetany is controlled or prevented by treatment, the secretion is unaffected (Peacock and Dragstedt, 1923). The secretion of bile and pancreatic juice is likewise diminished if the tetany is not controlled (Stoland, 1914). The disturbance in digestion may be so marked that undigested and partially decomposed meat may be removed from the stomach twelve and twenty-four hours after eating. Parathyroid tetany is little if at all influenced by reflexes from the gastro-intestinal tract, however, since its course is uninfluenced by previous section of the vagi and splanchnics (Palmer, 1920). When parathyroid tetany is prevented by calcium administration, the introduction of Ringer's solution, or a milk and lactose diet, digestion, absorption, and motility are apparently normal. The disturbances in the digestive tract are therefore an effect of the tetany and are not directly due to a decrease in the amount of parathyroid hormone liberated in the blood.

A depression in liver function plays no rôle in the genesis of parathyroid tetany, since this was not intensified in dogs by a previous Eck-fistula (Blumenstock and Ickstadt, 1924).

An increased permeability or lowered threshold for the excretion of calcium through the intestinal epithelium apparently exists after parathyroidectomy since the excretion of calcium into the intestine goes on even after the concentration of calcium in the blood has fallen below the normal (Salvesen, 1923).

Gastro-intestinal irritation will excite acute tetany in animals in latent tetany (Luckhardt and Compere, 1924) and this effect must occur through an alteration in the exchange of substances through the intestine since it cannot be explained on the basis of nervous reflexes. Constipation and meat feeding exert a similar augmenting influence on tetany.

BLOOD AFTER PARATHYROID EXTIRPATION. The literature dealing with the changes occurring in the blood following the removal of the parathyroids and coincident with the development of tetany is large and conflicting. The fall in the concentration of serum calcium from a normal of 10 to 12 mgm. per 100 cc. to one varying between 8 and 6 or 5 mgm. during the first 2 or 3 days is now well established. A decrease in the proportion of free or ionized calcium has been reported by some investigators (Trendelenburg, 1921; Pincus, Peterson, Kramer, 1926). An increase in the inorganic phosphorus in the blood occurs, but it is inconstant and varies considerably in amount (Greenwald, 1913, 1924; Salvesen, 1923; Gross and Underhill, 1922). It is possible that in some cases the changes observed may have been due to kidney injury, or even to ether anesthesia since this has been found to increase the inorganic phosphate of the blood in normal rabbits (Martland and Robson, 1924) and also in dogs (Stehle and Bourne, 1924). The decrease in the excretion of phosphorus however is consistent and it seems probable that the retained phosphorus is deposited in the tissues (Greenwald, 1913, 1924).

The statements that a condition of alkalosis develops after parathyroidectomy (Wilson, Stearns, Janney, 1915; Wilson, Stearns and Thurlow, 1915) is contradicted by the findings of a number of investigators (Greenwald, 1922, 1924; Underhill and Nellans, 1921; Hastings and Murray, 1921; Salvesen, 1923). The titratable alkali of the blood remains unchanged (Greenwald, 1924). However in the dog where hyperpnea is such a constant and early feature in parathyroid tetany, a slight alkalosis, due to removal of carbon dioxide, may occur (Cruickshank, 1924). That this hyperpnea may be an exciting factor in the tetany is not improbable since rebreathing in a closed chamber delays its onset or relieves it (Dragstedt and Sudan, 1926) and hyperpnea may induce temporary tetany even in normal animals and man (Grant and Goldman, 1920). A varying degree of hypoglycemia after thyroparathyroidectomy has been reported by Underhill and Blatherwick (1914) and Underhill and Nellans (1921). It seems possible that this may have been caused by the muscular exertion incident to the tetany since according to others (Hastings and Murray, 1921; Salvesen, 1923; Greenwald, 1924) the blood sugar remains within the normal range. The coagulability of the blood of tetany dogs may be markedly reduced (Simpson and Rasmussen, 1914; Dragstedt and Sudan, 1926), but this is not constant and in other experiments Simpson and Rasmussen (1916) denied that it occurred. An increase in the non-protein nitrogen of the blood in dogs parallel to the severity of the tetany is reported

by Florin (1925) but denied by Salvesen (1923) and Collip and Clark (1926). An increase was found following the tetany induced by pregnancy (Dragstedt, Sudan, Phillips, 1924).

TEMPORARY RELIEF OF PARATHYROID TETANY. A great variety of measures have been found effective in producing a temporary relief or recovery from the severe paroxysms of parathyroid tetany in animals. The intravenous injection of calcium lactate or calcium acetate produces a complete subsidence of all the symptoms of tetany (MacCallum and Voegtlin, 1908; Parhon and Urechie, 1907; Berkeley and Beebe, 1909). This relief is only temporary however and the tetany invariably recurs and death results (Marine, 1914; Voegtlin, 1917; Biedl, 1922). The concentrations of calcium salts (4 to 20 per cent) that have been commonly used for intravenous injection produce a nephritis (Blumenstock and Luckhardt, 1924) and this has no doubt complicated many of the early experiments. The oral administration of calcium salts was reported to be ineffective (Voegtlin, 1917). Salts of strontium, magnesium and barium are just as effective in causing this temporary relief as calcium (Berkeley and Beebe, 1909; MacCallum and Voegtlin, 1909; Dragstedt and Sudan, 1925, 1926; Swingle and Wenner, 1926). Temporary relief has also been secured by the injection of hypertonic sodium chloride solution (Joseph and Meltzer, 1910); by the injection of acids (Wilson, Stearns, Janney, 1915); by the injection of hypertonic sugar solutions, extracts of the thyroid, thymus, pancreas, testes and hypophysis, proteoses, peptones, amyl nitrite (Carlson, 1924) and other substances. Asphyxia may likewise temporarily relieve experimental tetany in dogs (Dragstedt and Sudan, 1926) and so also may the immersion of the animal in cold water and lowering the hyper-pyrexia (Boldyreff, 1908). Many of these effects may be accounted for by a decreased excitability of the nervous system and probably have no specific significance with regard to the essential cause and nature of parathyroid tetany. It is probable that lowering the body temperature by cold alleviates tetany indirectly by decreasing the hyperpnea and alkalosis. When hyperpnea does not exist cooling aggravates rather than relieves tetany.

PERMANENT CONTROL OF PARATHYROID TETANY AND DEPRESSION. Frouin (1908, 1910) reported on the possibility of preserving the life of completely thyro-parathyroidectomized animals by the administration of salts of calcium, an observation that seems to have had little influence on later work. A number of observers noted that dogs permitted to drink milk developed tetany much later and in milder form after para-

thyroidectomy than others and several reported that dogs (Breisacher, 1890) and rats (Blum, 1900) might survive on such a diet. The experience of many subsequent investigators has however demonstrated that whereas the tetany in dogs may be delayed in onset by allowing the animal to drink milk, it invariably develops and the animal dies (Marine, 1914; Paton and Findlay, 1916; Blumenstock and Ickstadt, 1924). MacCallum and Voegtlin (1909) gave milk by tube to dogs in amounts of from 500 cc. to 1000 cc. and Dragstedt and Sudan (1926) gave 500 to 2300 cc. without controlling tetany. The latter could not control the tetany nor prevent death in a pregnant dog with 1500 to 2900 cc. of milk daily.

The first definite statements to appear in the literature that parathyroid tetany could be controlled and that experimental animals could be regularly kept alive for long periods after complete extirpation of the parathyroid glands came from the experiments of Luckhardt and Rosenbloom (1922) and of Dragstedt (1922) and Dragstedt and Peacock (1923). Luckhardt and Rosenbloom reported that tetany appearing in thyro-parathyroidectomized dogs on a meat diet could be relieved by the slow intravenous injection of Ringer's solution in amounts of approximately 30 cc. per kgm. of body weight and further that by such injections repeated from four to eight times daily the animals regularly remained alive in good condition and free from tetany. Dragstedt and Peacock found that dogs if placed upon a meat-free diet and given from 50 to 100 grams of lactose per day or lactose plus 300 to 400 cc. of milk in the majority of cases would survive complete extirpation of the parathyroids indefinitely or for periods ranging from two months to two years. These observations were confirmed by Inouye (1924) who found that galactose was likewise effective.

Subsequently it has been found that this same survival can be secured by the careful and repeated applications of methods that previously had been found to afford only a temporary relief of tetany. The oral administration of calcium lactate (1.5 to 3 grams per kgm. of body weight per day) will afford such permanent relief from tetany and permit the indefinite survival of thyro-parathyroidectomized dogs (Luckhardt and Goldberg, 1923; Dragstedt and Sudan, 1926). A combination of a milk diet and the intravenous injection of small doses of calcium chloride (1 gram in 10 per cent solution) is also effective (Salvesen, 1923). It seems probable that previous investigators did not give calcium salts by mouth in large enough quantities nor at frequent enough intervals to be effective and that the amounts of such salts

given intravenously were so great as to produce a nephritis. Calcium lactate is not more effective when given orally than is calcium carbonate, calcium nitrate or calcium acetate whereas monobasic calcium phosphate is entirely ineffective (Compere and Luckhardt, 1924). Strontium lactate by mouth or intravenously, substituted in equal amount for the calcium in Ringer's solution, may preserve the life of parathyroidectomized dogs but with more difficulty than calcium (Dragstedt, 1925; Swingle and Wenner, 1926; Dragstedt and Sudan, 1926). A somewhat similar effect may be obtained by the oral administration of ammonium chloride (Boyd, Austin and Ducey, 1926) magnesium chloride (Luckhardt, Waud, Brannon, 1926), magnesium lactate (Wenner, 1926) and even by morphine sulphate (Sloan, 1926).

Of possibly different significance are the results reported following the oral administration of kaolin (Dragstedt, 1925; Dragstedt and Sudan 1926). In these experiments thyro-parathyroidectomized dogs were kept alive by a substance which is insoluble and must apparently exert its effect locally in the intestine. The beneficial effect of a milk diet is emphasized by Blum (1925) and attributed to the presence of the parathyroid hormone in the milk since similar effects in preserving the life of parathyroidectomized rats and cats are claimed to be obtained by feeding blood or serum. The efficacy of blood is questioned by the experiments of Beumer and Falkenheim (1926). There is no good evidence that the parathyroid hormone is active when given by mouth and the feeding of desiccated parathyroid glands does not relieve tetany (Waud and Luckhardt, 1925).

The above experimental facts illustrate clearly that the dog, and probably also the other experimental animals since these are uniformly more resistant, may under certain conditions survive as great a degree of parathyroid insufficiency as can be experimentally produced. It is probable that the usual fatal tetany of parathyroidectomy is due to an abrupt alteration in the blood and lymph dependent upon the sudden absence of parathyroid function but which can be controlled by measures which do not involve specific parathyroid therapy. After a varying period of four to six weeks the nervous system becomes so adjusted to the altered blood that tetany no longer results when treatment is stopped. That this seems to be the case is indicated by the fact that the serum calcium may be as low as 4 mgm. per 100 cc. three months after thyro-parathyroidectomy in the dog and the animal be free from symptoms (Dragstedt and Sudan, 1926; Blumenstock and Tweedy, 1927).

PHYSIOLOGICAL EFFECT OF THE PARATHYROID HORMONE. Although it is probable that a varying proportion of the active principle of the parathyroids together with much other material was present in the extracts made of the parathyroid glands by MacCallum and Voegtlin (1909), Berkeley and Beebe (1909), Hanson (1923, 1924), Massaglia (1921), Berman (1923) and perhaps others, nevertheless the first convincing demonstration of a physiologically active extract capable of affording successful substitution therapy and of raising the concentration of blood calcium was made by Collip (1925) and with it the first proof that the parathyroids perform their important function by means of an internal secretion. Extracts of the fresh parathyroids of animals (the ox) made with weak acid regularly contain varying amounts of the parathyroid hormone (Collip, 1925; Collip and Clark, 1925; Hanson, 1923, 1924, 1925; Davies, Dickens, Dodds, 1926; Hjort, Robison, Tendick, 1925; Berman, 1926; Schulten, 1925; Fisher and Larson, 1925, and Tweedy, 1926). It is a fairly stable substance, apparently a protein derivative (Collip, 1925) or a substance intimately associated with the protein fraction. When administered subcutaneously or intravenously in dogs it regularly prevents the appearance of tetany after parathyroidectomy, or relieves it after its appearance, and by its continued use the life of such animals may be preserved for three to six weeks after which they continue to live without treatment (Collip, 1925; Collip and Clark, 1925; Fisher and Larson, 1925). When given to dogs in active tetany, the symptoms are relieved and coincidently the lowered serum calcium is raised and the concentration of inorganic phosphorus in the blood decreased, if it is above the normal level (Collip, 1925, 1926; Hjort, Robison, Tendick, 1925; Berman, 1926). The hormone likewise increases the concentration of serum calcium in normal animals (Collip, Clark, Scott, 1925), the degree of hypercalcemia being directly proportionate to the amount of hormone given (Collip, 1926). This fact has made possible a physiological standardization of the amount of hormone in different extracts. A provisional unit of hormone strength has been arbitrarily defined (Collip, 1925) as 1/100 of the amount of extract that will produce an average increase of 5 mgm. in blood serum calcium in a normal dog of 20 kgm. weight over a period of 15 hours. Repeated injections of even small amounts of the hormone, at intervals of a few hours, into normal animals causes a much greater hypercalcemia than a single dose. The increase due to each injection is superimposed upon the high level of serum calcium due to a previous injection. A concentration of serum calcium as high as 23 mgm. per 100 cc. has

been obtained (Collip, 1925). Such repeated injections are dangerous and if continued invariably cause death (Collip, 1925; Fisher and Larson, 1925). Certain very characteristic changes have been described in these animals dying from overdosage of parathyroid hormone. The changes in the blood have been summarized by Collip (1925) as follows: "a great increase in viscosity; 15 to 20 per cent increase in osmotic pressure; increase in phosphates 100 per cent or more; increase in non-protein nitrogen as high as 400 per cent; increase in urea nitrogen as high as 400 per cent; increase in protein; diminution in halogen, 10 to 15 per cent; diminution in alkali reserve; diminution in blood volume 5 to 15 per cent." It is probable that some of the clinical symptoms as well as certain of the blood changes represent a secondary effect of the hypercalcemia and hyperphosphatemia. A condition closely resembling that seen in parathyroid hyper-dosage may be produced by the combined injection of both calcium chloride and acid sodium phosphate although either salt alone is ineffective (Collip, 1926). The bradycardia produced by excessive doses of parathyroid extract appears to be a result of the hypercalcemia (Edwards and Page, 1926) since it is relieved temporarily at least by solutions of magnesium sulphate (Matthews and Austin, 1927). These hypercalcemic animals have an increased tolerance to the depressant effect of magnesium salts (Matthews and Austin, 1927). At autopsy a metastatic deposition of calcium in the fundic glands of the stomach, in the colloid and stroma of the thyroid, and in the lungs, heart muscle, and kidneys is found (Hueper, 1927). A similar deposition of calcium is found in the kidneys of cats in whom tetany has been produced by pyloric occlusion (Zeeman, 1925).

It is not clear how the parathyroid hormone produces its characteristic effect on the blood calcium. There seems to be a direct quantitative relation between the amount of hormone liberated in the blood stream and the concentration of the blood calcium. Presumably under normal conditions the amount liberated suffices to keep the level constant in spite of variables in the way of absorption from the alimentary tract and excretion or withdrawal in special cases, such as pregnancy or lactation. The observation of Holt, La Mer and Chown (1925) that the quantity of calcium in the serum is greater than an aqueous solution of the same reaction and phosphate content could keep in solution is interesting. But the suggestion by Greenwald (1926) that the parathyroid hormone acts through keeping the calcium in solution in the blood is questioned by the fact that the serum calcium in completely parathyroidectomized dogs may be raised to its normal level by the oral administration of

calcium salts. The large amount of calcium required points to a defective absorption of this element as an important result of parathyroid deficiency. This defective absorption is in part remedied by parathyroid administration since the combined administration of parathyroid extract and of calcium lactate by mouth is more effective in raising the blood calcium than either measure alone. The lessened deposition of calcium in osteoid tissue may be directly due to the diminished concentration of calcium in the blood (Howland, 1923; Kramer and Howland, 1926), or to a loss of function of the osteogenetic cells. The removal of calcium phosphate from the bones when excessive doses of the parathyroid hormone are given and when sufficient calcium is not present in the diet (Greenwald and Gross, 1926) indicates a stimulation of the osteoclasts or other cells which are concerned in the removal of calcium from bone. It is not known if parathyroid overdosage will facilitate the removal of calcium from dead bone. However if adequate calcium is provided in the diet, parathyroid administration may facilitate its deposition in callus (Boez, 1919; Hueper, 1927).

PARATHYROID INSUFFICIENCY AND LATENT TETANY. The removal of two of the four parathyroid glands in the dog, cat or rabbit produces no nervous symptoms nor any changes in the blood. The extirpation of three glands however often causes transitory symptoms of more or less severe tetany and the serum calcium may fall to 7 mgm. per 100 cc. The animal may thereafter remain free from tetany except under certain special conditions to be described. The remaining parathyroid tissue undergoes a compensatory hypertrophy. If the four parathyroid glands of the dog be removed and the resulting tetany be controlled by any of the methods described above, after a varying period of four to eight weeks the animals may thereafter remain alive and free from tetany without treatment. The level of the serum calcium remains lower than normal (Dragstedt and Sudan, 1926; Blumenstock and Tweedy, 1927) and various chronic disturbances develop.

The appearance of bilateral cataracts was first noted by Erdheim (1906) in rats in which a chronic tetany had been produced by cauterizing the parathyroids. The condition has subsequently been observed in the rat by Possek (1907), R. Hayano (1920), and in the dog by W. Edmunds (1916), Luckhardt and Blumenstock (1923), and Dragstedt, Sudan and Phillips (1924). The cataract appears in the dog usually two to three months after the parathyroid deficiency has been produced, is always bilateral, and rapidly progressive. It is commonly punctate at first but later radiating opacities, a tripartite divi-

sion, and finally complete opacity of the lens develops. The mechanism of this cataract formation is unknown.

There is a defective deposition of calcium in certain osteoid tissues if the chronic parathyroid deficiency occurs during the growth period. There is a lessened deposition of calcium in the incisor teeth of rats (Erdheim, 1906, 1911; Preiswerk-Maggi, 1911, and Toyofuku, 1911) and also later a deficient calcification of the enamel, which together with erosions and disturbances in the structure of the enamel epithelium causes the teeth to become easily broken and carious. A defective deposition of calcium in the callus following fractures occurs in chronic parathyroid deficiency in rats (Canal, 1910) and cats (Morel, 1909, 1910). The callus may resemble that found in rickets (Erdheim, 1911). The deposition of calcium in the skeleton goes on imperfectly and changes have been described which in some respects resemble those of rickets and osteomalacia (Erdheim, 1911).

Often dogs with chronic parathyroid deficiency become deaf (Dragstedt and Sudan, 1926) of interest in view of the finding of Frey and Orzechowski (1917, 1920) of symptoms of chronic tetany in patients with otosclerosis.

In severe parathyroid deficiency in the dog a progressive cachexia but without tetany may occur, which differs from that of thyroid deficiency in that it can be prevented and controlled by calcium therapy.

Experimental animals, suffering from chronic parathyroid deficiency, may develop acute often fatal attacks of tetany under a variety of conditions. *Oestrus* will regularly precipitate a more or less severe tetany in dogs (Luckhardt and Blumenstock, 1922; Dragstedt, Phillips and Sudan, 1923). Similarly acute tetany frequently recurs at each menstruation in women with latent tetany due to parathyroid insufficiency (Meinert, 1898; Lundborg, 1904). The phenomena appear to be analogous in which case it is evident that the changes in the uterine mucosa are more significant than ovulation as an exciting factor. Ovulation, in the dog, practically coincides with oestrus while in primates it takes place in the intermenstrual period. The mechanism of this oestrual tetany is not clear. Removal of the ovaries does not relieve it (Luckhardt, 1924). It is not brought about by an increase in metabolism at this time as suggested by Paton (1924) since the metabolic rate remains within normal limits during oestrus in dogs (Kunde, 1923) and during the menstrual period in women (Blunt and Dye, 1921). Increasing the rate of metabolism by thyroid feeding does not excite tetany in dogs with parathyroid deficiency (Dragstedt, 1925). The

concentration of blood calcium is more variable during menstruation in women than during the intermenstrual period (E. D. Allen, 1927). In 77 cases Rittmann (1924) found that the blood calcium level remained unchanged in 13, that it was increased in 31, and decreased in 33, during menstruation. Determinations of the level of the serum calcium have not been made during the tetany of oestrus in dogs, but this tetany may be relieved and controlled by lactose and milk feeding, calcium administration, or the intravenous injection of Ringer's solution. The evidence suggests an antagonistic influence of the ovaries and parathyroids on some phase of metabolism.

Pregnancy not only increases the severity of the tetany and shortens the life of recently parathyroidectomized dogs, but it regularly transforms a latent into an active and often fatal tetany. Dogs and rats, in which a chronic parathyroid deficiency has been produced by partial extirpation, although free from tetany for months, develop manifest tetany at each pregnancy (Halsted, 1896; Vassale, 1897; Adler and Thaler, 1908; Massaglia, 1913). This tetany develops during the latter part of pregnancy and usually just before and after delivery. The early part of pregnancy may exert a beneficial influence. The tetany is relieved by emptying the uterus, by the oral administration of calcium lactate or the intravenous injection of Ringer's solution (Luckhardt and Rosenbloom, 1922; Dragstedt, Sudan, Phillips, 1924). The mechanism by which pregnancy excites tetany is not definitely known. It is quite evident that the fetal parathyroids of dogs and rats cannot function vicariously for the mother as is the case with the internal secreting function of the fetal pancreas (Carlson and Drennan, 1911; Carlson, 1913). Late pregnancy likewise excites tetany in women with parathyroid deficiency (Frankl Hochwart, 1891; Thomas, 1895) but it does not appear to alter the course of parathyroid tetany in the cat (Carlson, 1913). The hypertrophy and hyperplasia of the parathyroids during pregnancy (Seitz, 1923) is similar to that produced by diets deficient in calcium (Marine, 1913; Luce, 1923). It is evident that pregnancy produces an unusual abstraction of calcium and, if the intake is deficient, the mother loses body calcium as evidenced, in the rat, by analyses of total maternal calcium, by roentgenograms of the bones, and by specific decalcification of the teeth (Macomber, 1927). The calcium content of the fetus is normal. There is no evidence however even when the diet is low in calcium, that pregnancy lowers the concentration of that element in the blood. The calcium concentration of the blood of normal women in early or late pregnancy is not different

from that of non-pregnant women of the same age (Jansen, 1918; Lamers, 1918). It is possible however that the mechanism for maintaining a constant level of serum calcium, which would compensate for the specific removal in pregnancy, is defective after partial or complete parathyroidectomy. Of interest in regard to the etiology of the tetany of pregnancy is the observation of Fromme (1906) that acute tetany may be precipitated in partially parathyroidectomized animals by the injection of placental extract.

Lactation is a more effective stimulus than either oestrus or pregnancy in producing an acute exacerbation of tetany in the dog with chronic parathyroid deficiency. The severity of the tetany is to some extent directly proportionate to the amount of milk withdrawn (Dragstedt, Sudan, Phillips, 1924). This tetany can be entirely relieved and controlled by the intravenous injection of Ringer's solution or the oral administration of calcium lactate. There is no evidence that the serum calcium of normal animals is reduced by lactation, but, in the rat lactation produces a much greater drain on the maternal calcium than does pregnancy (Macomber, 1927). It is possible therefore that pregnancy and lactation may act in a similar way as exciting factors in parathyroid tetany.

Other factors which may precipitate an acute attack of tetany in animals in which it is latent are: muscular exertion (Massaglia, 1907), the administration of certain toxic chemicals such as atropine, morphine, ergot, tuberculin and phosphorus (Rudinger, 1908; Dragstedt, Phillips, Sudan, 1923), guanidine and methyl guanidine (Paton, Findlay, Watson, 1916), meat feeding (Berkeley and Beebe, 1909), and especially putrid meat (Luckhardt and Rosenbloom, 1922; Dragstedt, Phillips, Sudan, 1923), constipation (Dragstedt, Phillips, Sudan, 1923), high temperatures (Boldyreff, 1908), gastro-intestinal irritants such as sulphur, cascara sagrada and crotin oil (Marine, 1914; Luckhardt and Compere, 1924), infections (Dragstedt, Phillips, Sudan, 1923), and possibly many others.

Parathyroid deficiency renders the dog much less resistant to a variety of toxic substances. Guanidine, methyl guanidine, trimethylamine, histamine and physostigmine, in amounts too small to affect normal animals, will cause a severe intoxication (Dragstedt, Phillips, Sudan, 1923). The lessened resistance to infection is due apparently to a defective ability to produce antibodies (Carlson, 1913).

THE PARATHYROID GLANDS AND CALCIUM METABOLISM. It is clearly established that the parathyroid glands play an important rôle in the

maintenance of the normal level of blood calcium and probably in the transfer of calcium from the alimentary tract and its deposition in the osteoid tissues during growth or repair. This function is performed through the agency of a specific internal secretion. The evidence may be summarized as follows:

1. The partial or complete absence of parathyroid function results in a diminution in the concentration of calcium salts in the blood (MacCallum and Voegtlin, 1909; Hastings and Murray, 1921; Salvesen, 1923, and others). A partial removal of the parathyroids may produce a slight fall, a complete extirpation, a much more marked fall in blood calcium (Salvesen, 1923). The calcium in the blood serum of normal dogs averages 10 to 11 mgm. per 100 cc. while that in completely parathyroidectomized animals may fall as low as 5 or even 4 mgm. All variations are found but commonly the figures shortly after complete parathyroidectomy range between 5 and 7 mgm. per 100 cc. of serum.

2. The partial or complete absence of parathyroid function causes a lessened excretion of calcium from the body. The early statement of MacCallum and Voegtlin (1909) that there occurs an increased excretion of calcium through the intestines after parathyroidectomy was not corroborated by Cooke (1910) and the recent studies of Greenwald (1925) on dogs indicate that parathyroid deficiency causes rather a decreased calcium excretion in the feces and urine. This decreased excretion appears to be a direct result of the decrease in blood calcium and is not due to an impermeability of the intestine to calcium salts. Calcium salts given by mouth to parathyroidectomized dogs are absorbed (Luckhardt and Goldberg, 1923; Hjort, 1925), and injected calcium is rapidly excreted (Salvesen, 1923). The observation that this excretion persists even when the concentration of blood calcium is less than normal may indicate that the intestinal epithelium has a lowered threshold or increased permeability to calcium.

3. The partial or complete absence of parathyroid function causes a lessened deposition of calcium in the osteoid tissues during periods of growth or repair. Growing rats and dogs, after partial parathyroidectomy, deposit enamel, dentine, bone, and callus which is poorer in calcium than in the normal animal (Erdheim, 1911; Iselin, 1908; Toyofuku, 1911; Canal, 1909; Morel, 1911, and Korenchevsky, 1922). Following destruction of the parathyroids in a rat by cauterization, Erdheim (1911) found that a calcium-poor layer of dentine was laid down. Then by implantation of two parathyroids from a second young rat a layer of dentine with normal calcium content was deposited and on

removal of the transplants a second layer of calcium-poor dentine occurred.

4. Experimental conditions which bring about a decreased supply of available calcium to the organism are found to produce an hypertrophy and hyperplasia of the parathyroid glands. Marine (1913) found that hens fed on a calcium-poor diet developed a marked enlargement and hyperplasia of the parathyroids and Luce (1923) observed similar changes in the parathyroids of rats when the intake of calcium was diminished. A partial parathyroidectomy in rats or dogs produces a latent or chronic tetany and an hypertrophy of any remaining parathyroid remnants (Halsted, 1907; Pepere, 1908; Tauberg, 1914). It is probable that this is a compensatory hypertrophy to meet the need of the organism for more of the parathyroid hormone. Such parathyroid deficiency is accompanied by a lowered blood calcium and this may represent the stimulus for increased activity and later hypertrophy of the remnant. Pregnancy and lactation are likewise accompanied by a similar hypertrophy of the parathyroids and in these conditions there is an unusually great withdrawal of calcium. However neither pregnancy nor calcium-poor diets decrease the concentration of serum calcium in normal animals and it is not known whether or not calcium administration will prevent the compensatory hypertrophy of a parathyroid remnant. It is significant that chronic guanidine poisoning does not produce an hypertrophy of the parathyroids (Bayer and Form, 1924).

5. Certain diseases characterized chiefly by a disturbance or failure in the normal calcification of bone are likewise characterized by a resultant hypertrophy of the parathyroids. Thus hypertrophy of the parathyroids has been observed in *rickets* (Erdheim, 1914; Weichselbaum, 1914), in *osteomalacia* (Erdheim, 1907; Strada, 1909; Th. Bauer, 1911; Todyo, 1912; C. Ritter, 1920), in *senile osteoporosis* (Todyo, 1912; Ritter, 1920), in *ostitis fibrosa* (Maresch, 1916; Hoffheinz, 1925), in *ostitis deformans* (Todyo, 1912), in von Recklinghausen's *multiple fibrocystic disease of bone* (Hoffheinz, 1925; Mandl, 1926), and perhaps others. In no case has it been demonstrated that the disorder has been caused by hypertrophy or hyperfunction of the parathyroids. On the other hand, although these diseases are for the most part of uncertain etiology, it is probable that the observed changes in the parathyroids are produced by the disease or by the alteration in calcium metabolism which results from the disease. Erdheim (1914) found that the hypertrophy of the parathyroids occurring in spontaneous rickets in rats did

not develop until the disturbance in the skeleton was marked and further that the hypertrophy receded with the healing of the disease. In this connection it is interesting that irradiation, which has been shown to have a curative effect in rickets, causes hypertrophy of the parathyroids in rabbits and an increase in the blood calcium (Grant and Gates, 1924). This apparently contradictory finding is difficult to understand. The irritation leading to parathyroid hypertrophy in these diseases is apparently nor primarily a decrease in blood calcium or phosphorus since with the exception of rickets the concentration of these elements is reported to be normal.

6. The administration of calcium salts to animals in which a characteristic disease syndrome (parathyroid tetany or depression) has been produced by absence of parathyroid function, to a very large extent corrects this disturbance. MacCallum and Voegtlin (1909) demonstrated that an animal in violent parathyroid tetany could be temporarily restored to a normal state by the intravenous injection of certain soluble calcium salts (calcium acetate and lactate). This fact has been abundantly confirmed. Luckhardt and Goldberg (1923) demonstrated that by the daily oral administration of calcium lactate in large amounts (1.5 grams per kgm. of body weight) totally thyroparathyroidectomized dogs could be kept alive in a nearly normal state and free from tetany for indefinite periods (1 to 2 years). This finding was confirmed by Dragstedt and Sudan (1926). This oral administration of calcium salts raises the concentration of the blood calcium (Luckhardt and Waud, 1925; Hjort, 1925) but it rapidly falls again if the administration in large doses is stopped. The calcium does not however prevent the appearance of cataract in thyroparathyroidectomized dogs and it is not known if it brings about the normal deposition of calcium in bone, callus, dentine and enamel.

7. The administration of a physiologically active extract of the parathyroids to parathyroidectomized animals raises the blood calcium to its normal level (Collip, 1925) and excessive doses to normal or parathyroidectomized animals raise the blood calcium to abnormally high values (18 to 23 mgm. per 100 cc. serum) (Collip, Clark, Scott, 1925). This hypercalcemia may be obtained even when the diet is deficient in calcium and it leads to an increase in calcium excretion (Greenwald and Gross, 1926). It is probable that when insufficient calcium is present in the food the administration of the hormone may cause its removal from the bones. Excessive amounts of the parathyroid hormone produce an abnormal deposition of calcium in the fundic

glands of the stomach, in the thyroid, lungs, heart muscle and kidney (Hueper, 1926).

THE PATHOGENESIS OF PARATHYROID TETANY. The greater part of the experimental work on the parathyroid glands has been devoted to attempts to analyze the nature of parathyroid tetany and to demonstrate the causal relationship of the changes in the blood and nervous system that bring it about. It is clear that the hyper-irritable state of the peripheral nerves and probably also the convulsive phenomena attributed to stimulation of the midbrain, cerebellum, and spinal cord, are in turn due to an alteration in the blood and lymph. This is indicated by the fact that the hyper-irritability can develop in peripheral nerves that have been previously sectioned. The perfusion of blood from a dog in active tetany through the isolated extremity of a normal dog produces an increased electrical excitability of the peripheral nerves in the perfused leg (MacCallum, 1913; Jacobson, 1923). The nature of the alteration in the blood after parathyroidectomy which causes tetany has not been satisfactorily determined. A great many explanations have been offered but only two of these will be analyzed in any detail.

THE CALCIUM DEFICIENCY THEORY OF THE GENESIS OF TETANY. Following the lead of MacCallum and his associates an attempt has been made to attribute the hyper-irritability of the nervous system in parathyroid tetany to a relative decrease in the concentration of calcium in the circulating blood. This theory is supported by the following main lines of evidence. The irritability of isolated muscles and nerves suspended in various balanced physiological salt solutions may be increased by lowering the concentration of calcium in proportion to that of sodium in these mixtures (J. Loeb, 1899, 1900). When tetany appears following thyro-parathyroidectomy the serum calcium is usually and in the experience of many investigators always reduced from a normal of about 10 mgm. per 100 cc. to below 7, while the concentration of sodium and potassium is unchanged (MacCallum and Voegtlin, 1909; Hastings and Murray, 1921; Salvesen, 1923). The extent of lowering of the blood calcium corresponds roughly with the degree of severity of the tetany in recently operated animals. The inactivation of the circulating calcium in normal animals by the intravenous injection of sodium citrate, oxalic acid, or sodium oxalate is accompanied by muscular twitchings and convulsions similar to those of tetany, and these may be relieved by the injection of soluble calcium salts in sufficient quantity to restore the equilibrium (Loeb, 1901;

MacCallum and Vogel, 1913; Trendelenburg, 1921). The perfusion of blood from a dog in active tetany into the isolated leg of a normal dog increases the electrical excitability of the nerves in the perfused leg (MacCallum, 1913; Jacobson, 1923) and a similar effect may be obtained by perfusing normal blood from which the calcium has been partly removed by dialysis (MacCallum, Lambert and Vogel, 1914). The intravenous introduction of certain soluble calcium salts relieves for a time all the signs and symptoms of tetany (MacCallum and Voegtlin, 1909; Berkeley and Beebe, 1909, etc.). Corresponding to this relief the blood calcium returns to its normal level and on the return of tetany is found again at a low level (Salvesen, 1923). The oral administration of calcium lactate, carbonate or acetate relieves tetany and when given daily permits completely parathyroidectomized dogs to live for months or years free from tetany (Luckhardt and Goldberg, 1923; Dragstedt and Sudan, 1926). The amounts of these salts that are effective when given by mouth raise the blood calcium toward the normal level (Luckhardt, 1925; Hjort, 1926). The administration of an active preparation of the parathyroids (Collip, 1925) relieves parathyroid tetany and effects a simultaneous proportionate elevation of the blood calcium. Excessive amounts of this preparation increase the serum calcium above the normal concentration and bring about symptoms of weakness and depression in some respects the antithesis to those of tetany (Collip, 1925; Collip, Clark, Scott, 1925).

The facts outlined above would appear well nigh conclusive in proving that parathyroid tetany at least is due to the diminished blood calcium, were it not for certain observations which at present are not readily explained on this basis. The withdrawal of blood from a dog in violent tetany and the substitution of an equivalent amount of a physiologic salt solution free from calcium affords a distinct although temporary relief. However bleeding alone causes a relief but this is accompanied by an increase in the serum calcium (Swingle and Wenner, 1926). The intravenous injection of Ringer's solution causes a complete relief of tetany and when repeatedly applied a permanent control (Luckhardt and Rosenbloom, 1922). This relief is not however regularly accompanied by a return of the lowered blood calcium to its normal level (Blumenstock, 1926). A similar relief may be obtained by the intravenous injection of calcium-free Ringer's solution (Luckhardt and Rosenbloom, 1922) or a modified Ringer's solution in which strontium has been substituted in place of calcium (Dragstedt and Sudan, 1926). It is probable that many of the procedures that may cause a temporary

relief of tetany and that when repeatedly and judiciously applied may preserve the life of parathyroidectomized animals act through a depressant effect on the nervous system and do not elevate the blood calcium. This appears to be the case in the treatment of tetany with magnesium chloride (Luckhardt, Waud, Brannon, 1926), with morphine sulphate (Sloan, 1926), and possibly also with strontium salts (Berkeley and Beebe, 1909; Dragstedt and Sudan, 1926). It does not follow from this that the lowered calcium is not the causative factor in tetany since its irritant effect may be counterbalanced by the depression induced. However after the first critical period which follows immediately on complete parathyroidectomy is past and the method of tetany control employed relaxed, tetany may occur from time to time and the serum calcium be higher during the period of tetany than at others when the animal is symptom-free. After four or five months the animal may remain entirely tetany-free with a serum calcium as low as 5 or even 4 mgm. per 100 cc. Nevertheless a severe or fatal attack may be induced by oestrus, pregnancy, or lactation. The daily administration of cod liver oil for two weeks before operation is reported to increase the length or life of thyro-parathyroidectomized dogs for long periods (average of 32 days), tetany remains absent, but the blood calcium falls as in untreated animals (Jones, 1926). Conversely parathyroidectomized rabbits treated with parathyroid extract may die in tetany and yet the blood calcium be within the normal range (Collip, 1926). These animals have a very high blood phosphorus.

It is not readily evident how many of the factors, which are known to induce tetany in animals with varying degrees of parathyroid deficiency, can lower still further the level of blood calcium. The effect of pregnancy and lactation may be explained on the withdrawal of maternal calcium and the failure of an adequate compensatory mechanism in the absence of parathyroid function. The effect of oestrus and menstruation is not so readily understood. It is however in respect to the alimentary tract that the situation seems particularly confusing. Meat feeding, constipation, and gastro-intestinal irritation may provoke severe tetany in susceptible animals. Conversely meat-free diets containing lactose, galactose, or even kaolin may entirely prevent parathyroid tetany.

A dog, whose life has been preserved after thyro-parathyroidectomy by adequate treatment for the first critical period of six to eight weeks, may thereafter remain free from tetany for long periods on a diet of bread and water. It may then be thrown into violent tetany by the

feeding of meat. It is thus evident that there must be something in meat or produced by the meat ingestion which induces tetany. The difference between the calcium content of the two diets is not great enough to account for the effect of meat feeding on this basis. It is more probable that on the meat diet some substance is absorbed which causes tetany, but whether this is phosphate which lowers the blood calcium (Greenwald, 1926), a nitrogenous compound which lowers the proportion of ionized calcium in the blood, or a toxin which provokes tetany in a susceptible animal, is not known. The interpretation that parathyroid tetany is dependent upon the absorption of a tetany toxin from the alimentary tract which is prevented from forming by lactose diets or from being absorbed by the presence of kaolin (Dragstedt) is questioned by the finding that this tetany is not aggravated by the production of an Eck-fistula (Blumenstock and Ickstadt, 1924). There is evidence that the increase in acidity of the intestinal content as a result of lactose feeding favors the absorption of calcium (Bergeim, 1926) but it seems hardly possible that enough calcium from these diets can be absorbed to prevent tetany. A minimum of 1.5 grams of calcium lactate per kgm. of body weight per day was required by Luckhardt and Goldberg (1923) or an equivalent of 0.2727 gram of calcium oxide per kgm. of body weight per day given as calcium lactate, chloride or glycerophosphate (Hjort, 1925). However, it is interesting that lactose, galactose or kaolin feeding, which ameliorate tetany, produce an acid intestinal content while meat feeding or constipation which aggravate tetany produce an alkaline one. It is probable that one of the factors which account for the superiority of human milk over cow's milk in the treatment of tetany is its greater content of lactose (Dragstedt and Sudan, 1926).

THE INTOXICATION THEORY OF THE GENESIS OF TETANY. It is difficult to account for the great variation in symptoms displayed by different species and by different animals of the same species after thyroparathyroidectomy solely on the basis of a lowered blood calcium. It is possible that this may increase the irritability of nerves and muscles but many animals are sluggish and depressed from the start and never give evidence of an excited state. That the signs and manifestations of parathyroid tetany are due to the accumulation of toxic substances in the blood which affect the nervous system is one of the oldest theories of tetany. It was perhaps suggested through analogy with the liver, also an outgrowth from the alimentary tract, which has a detoxicating function and by the gross similarity between parathyroid tetany and

the nervous manifestations in Eck-fistula animals and the aggravating effect of a meat diet in both conditions (Carlson and Jacobson, 1910). Recent work has been concerned with attempts to demonstrate the existence of such a toxemia and to secure evidence as to the nature and source of the tetany toxins. Much of this evidence is indirect and may be summarized as follows. The relief and control of parathyroid tetany which may be obtained by bleeding and the infusion of salt solution, the intravenous injection of Ringer's solution, calcium-free Ringer's solution, 0.9 per cent sodium chloride solution, or strontium-containing Ringer's solution, may be attributed to the dilution and rapid removal by diuresis of a soluble tetany poison from the blood stream. With the exception of the strontium solution there is no evidence that these fluids exert a depressant or drug action on the nervous system or that they raise the calcium concentration of the blood. The prevention of parathyroid tetany and the preservation of life of parathyroidectomized dogs by meat-free diets together with lactose, or galactose, and milk may be attributed to the lack of production of tetany poisons in the alimentary tract when bacterial proteolysis is checked. The effect of kaolin may be explained as due to the prevention of absorption of these tetany poisons because of the adsorbent effect of the kaolin on the toxin.

The ameliorating effect of removal of the colon on parathyroid tetany in dogs (Oldberg and Ivy, 1925) may be interpreted as due to the removal of an important source of tetany poisons, and indeed it is difficult to see how it can be explained in any other way.

The reported production of tetany in normal dogs by irritation of the duodenal mucosa with chloroform (Spodolini, 1926) may also be attributed to increased absorption of a tetany poison from the intestine. The aggravating effect of meat feeding, putrid meat, constipation and gastro-intestinal irritation on latent tetany animals may be attributed to an increased production and absorption of tetany toxins from the alimentary tract under these conditions.

Attempts to secure direct evidence of the presence of a toxemia after parathyroidectomy have not been very successful. The urine of dogs in active parathyroid tetany is more toxic than normal urine (Laulanie, 1894) and increased amounts of guanidine, methyl-guanidine, trimethylamine, and methyl cyanamide are reported to be present (W. F. Koch, 1912, 1913, 1916; Burns and Sharpe, 1916). Increased amounts of guanidine are likewise reported to be present in the blood of tetany animals (Burns and Sharpe, 1916). However the chemical methods

available for the determination of guanidine in the blood and urine are not satisfactory, a fact recognized by Paton (1924), and the method of Greenwald (1924) failed to show any increase in these substances in parathyroid tetany animals. The administration of guanidine and methyl-guanidine to normal animals (cats, guinea pigs, rats and dogs) produces a symptom complex very much like that of parathyroid tetany. That the symptoms are identical as stated by Paton (1924), is not confirmed by other investigators (Dragstedt, Phillips, Sudan, 1923; Ivy, 1924; Nicholas and Swingle, 1924). An increase in the electrical excitability of the peripheral nerves is regularly observed in chronic guanidine poisoning (Paton and Findlay, 1916; Frank, Stern, Nothmann, 1921; György and Vollmer, 1922). Small doses of guanidine produce merely an increase in the electrical excitability of the nerves while larger amounts produce tonic and clonic convulsions, which, especially in the cat, resemble those of parathyroid tetany. There is the same change in nitrogen metabolism (Burns, 1917), a similar hypoglycemia (Watanabe, 1918), and a similar decrease in liver glycogen (Underhill and Blatherwick, 1911–1922; Bayer and Form, 1924). Very small doses of guanidine or methyl guanidine aggravate the symptoms of tetany when already present or hasten their appearance in recently operated animals (Paton, 1924). Likewise such amounts, too small to affect normal animals, may cause severe convulsions in dogs with chronic parathyroid deficiency (Dragstedt, Phillips, Sudan, 1923). These dogs are however much more susceptible to many different poisons than normal animals. The development of cataract and defective calcification of dentine and enamel, so characteristic of chronic tetany in rats, is not seen in chronic guanidine poisoning (Bayer and Form, 1924), nor does such poisoning produce hypertrophy of the parathyroids. While most recent observers are agreed that parathyroid tetany is regularly accompanied by a fall in the concentration of blood calcium, the evidence with regard to this point in guanidine intoxication is conflicting and it seems probable that a decrease comparable to that seen in tetany does not occur. Bayer (1922) using the biological method of Trendelenburg (1921) reported a decrease in the ionized calcium in the serum of guanidine poisoned rabbits, cats, and guinea pigs, but not so great as in parathyroid tetany and others using chemical methods for the estimation of the total serum calcium (Kramer and Howland, 1920) found no decrease in normal animals poisoned with guanidine (Nelken, 1923; Salvesen, 1923; Collip and Clark, 1926). Dragstedt and Sudan (1926) found that dogs that had been kept alive after thyro-parathy-

roidectomy by treatment and which were tetany-free although the blood calcium was lower than normal, did not suffer a further decrease on being poisoned with guanidine. The evidence on the effect of calcium administration in guanidine poisoning is likewise contradictory. While the ameliorating effect of calcium on parathyroid tetany is easily demonstrated, a comparable effect on guanidine poisoning is not found (Klinger, 1921; Bayer, 1921). Conversely the administration of an active parathyroid extract does not protect dogs poisoned with guanidine (Collip and Clark, 1925).

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PHYSIOLOGICAL RESPONSES TO HEAT

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Temperature variations affect nearly every physical, chemical and biological process; often the effect is profound. It is therefore impossible to outline here systematically the effects on various biological processes, or upon various species of animals; hence the presentation attempted will be limited to the comparatively narrow field of the changes that occur in mammals as the result of exposure to environments of high temperature, though reactions to low temperatures will also often be referred to, in order to obtain a more comprehensive point of view. Even in this field attention will be directed mainly to the conditions existing in man, and only to those in other mammals in so far as they help to elucidate the mechanisms concerned. For similar reasons some observations made on cold-blooded animals will also at times have to be referred to. The term response will have to be interpreted liberally, since at the present time it is often impossible to distinguish between changes due to the direct physical effects of change of temperature, to variations in chemical activity secondary to these physical changes, or to alterations in glandular, muscular or nervous processes secondary to either or both of these. The whole subject is unfortunately lamentably complicated and little understood; indeed we have advanced little beyond the views expressed by Claude Bernard in 1876 in his masterly lectures on *La Chaleur Animale.*

No attempt will be made here to present a complete bibliography; fortunately this is not a serious omission since excellent bibliographies are contained in recent articles by Isenschmid, Freund, Schade and Kestner in Bethe's Handbuch der normalen und pathologischen Physiologie (1926). The literature in relation to temperature control was also reviewed in 1921 by Barbour and excellent summaries of the older literature are given by Pembrey (1898 and 1913) by Lefèvre (1911), as well as by Richet (1898), who has himself done so much of the pioneer work in this subject. Sundstroem (1927) has recently discussed tropical climates.

EXCHANGE OF HEAT. The heat to which an individual may react may be of external origin or may be generated internally by metabolic processes. Since the origin of homoiothermic animals, organisms have had to adapt themselves to both sources of heat so that the reactions involved must be very primitive. As a warm-blooded animal normally leads an active life and therefore must eliminate superfluous heat, external warmth must be defined as any environmental factor which renders more difficult normal heat loss; hence the term need not imply the existence of an external temperature above that of the body. Heat loss by radiation, convection, and conduction varies not only with the temperature difference between the surface of the body and the surrounding air, but is also much affected by the thermal conductivity and capacity of other substances in contact with the skin, as well as by the conductivity of the skin itself. Thus the differences in the degree of heat exchange of the body to water and to air are due to these latter factors, since water has a thermal conductivity about 25 times, and a capacity about 4,200 times that of dry air. Consequently water at 30° to 34° feels cool while air at the same temperature feels very warm. The former gives a very high, the latter a very low heat loss, unless compensation is attained through physiological reactions. According to Lefèvre (1911) the conductivity of the dermis varies between values $\frac{1}{4}$ and $\frac{1}{2}$ that of water.

In air heat loss occurs through radiation, and especially through conduction, convection as well as by evaporation of water, so that air movement and humidity are of great importance. Dry air at a temperature far above that of the body does not necessarily cause any rise of deep body temperature, nor even of surface temperature, since the loss of heat through evaporation of water is often able to balance that gained by radiation and conduction as well as that produced in metabolism. On the other hand air saturated with moisture, even though many degrees below the body temperature, may not be able to remove as much heat by radiation, conduction and convection as the body produced in metabolism, so that a rise of rectal temperature results. If, however, the air is kept in rapid movement, so as to utilize its heat capacity and conductivity to the utmost, the maintenance of a balance may still be possible. Should a rise of body temperature occur, it is apt to be progressive, since the metabolism and heat production are increased by the rise in temperature more than the rate of heat loss. A new equilibrium at a higher body temperature level can therefore only be established within a relatively narrow temperature range, and even then only through physiological adjustments.

Attempts have been made to express the cooling power of the air in more exact terms. As the result of experiments on the loss of heat through isolated moistened sheep skin, Phelps (1923) devised a formula combining the various factors into one unit, but the data were artificial, the formula over emphasized evaporation, and its use gives results which do not correlate with physiological experience. Hill, Griffith and Flack (1916) introduced the idea of the direct measurement of the cooling power of the atmosphere for dry or wet objects at about body temperature, and devised an instrument which they termed the kata-thermometer. This is simply a thermometer whose rate of cooling after artificial warming is measured and expressed in calories per square centimeter per second. While it is an advance to consider temperature in terms of the rate of exchange of calories, the instrument is inadequate and fails to take all the factors into consideration. The cooling power is measured for a body of 36.5°C., but the actual loss of heat in man is from a skin surface of 33° to 35°; any individual is abnormal whose surface temperature is as high as 36.5°. The kata-thermometer may be used to measure the cooling rate of a dry or a wet body, but the human body does not normally correspond with either; it has a partly moist surface, and it remains uncertain how these dry and wet readings should be combined. The kata-thermometer has been developed into the recording comfortmeter of Schuster (1923) and similar methods of measuring cooling power have been described by Schade (1926) and by Thilenius and Dorno (1925). The last of these have improved the method by employing 33° as the critical temperature instead of 36.5°; they employ a copper mass instead of alcohol. Such instruments are affected not only by the dry bulb temperature, but also by the air movement, and they demonstrate the cooler condition existing when the air is moved by wind or by a fan than when it is still, even though the actual temperature is unchanged. Nevertheless, the values obtained with Hill's kata-thermometer do not always agree with the sensation experienced (Vernon, 1923; Ingels, 1924).

Very important work on this subject, carried out in Pittsburgh, has recently been published in a series of papers by Houghton and Yagloglou (1923a, b; 1924), Yagloglou and Miller (1924, 1925), Yagloglou (1926). These workers seem to have succeeded in expressing in one term the various factors concerned in heat loss, and, since their papers may not be readily available to some interested in the subject, two of their figures will be reproduced here. They estimated comparable room conditions by the effect produced on sensation. Human subjects remained for a

time in a room of a given temperature with a high humidity but no air movement; they then passed into another room of known, but different, humidity and dry bulb temperature. The subjects passed back and forth from one room to the other until the conditions in the second room had been adjusted to give exactly the same temperature sensation as in the first. If sensations of temperature depend, as seems probable, on the setting up of different rates of heat loss from the skin, the absence of any change in temperature sensation should be a good index of comparable conditions. In this way various conditions of dry and wet bulb temperature were investigated with different rates of air movement. The results are presented in the form of curves showing comparable conditions which produce similar sensations. A measurement of the cooling power of any environment for man is thus obtained, and is expressed in terms of an "effective temperature," that is, the temperature of a room saturated with moisture and without air movement, in which the sensations experienced are similar to those observed under some variable conditions, the cooling power of which is to be determined. Such data do not take into consideraton such factors as radiant heat from a fire, but the results obtained are satisfactory for ordinary conditions and allow the humidity factor to be considered rationally. The data of Yagloglou and his co-workers demonstrate that in a warm environment, where the subject sweats, the wet bulb temperature and the air movement are the dominant factors, as Haldane originally pointed out (1905), but that under more common and cooler conditions, when the subject is not sweating, the dry bulb temperature is the more important factor. Air movement has also an immense effect. Hill's kata-thermometer is a very useful instrument for determining the latter value, since it measures not only regular but irregular air movement, and it is employed by the Pittsburgh workers for this purpose (Ingels, 1924). Convenient tables for such calculations are given by Hargood-Ash and Hill (1923) p. 41).

The figures reproduce the nomograms given by Yagloglou and his coworkers to represent comparable conditions for subjects with light clothing (fig. 1), and when stripped to the waist (fig. 2), the latter representing what they call the "basic scale." The values were given originally in the Fahrenheit temperature scale and the air movement in feet per second; some centigrade values have been added to the diagrams to facilitate scale conversions. If the dry bulb temperature be read on the left and the wet bulb on the right, the line joining them crosses the "effective temperature scale." Under ordinary room conditions no

great error will result from neglecting the air movement and reading the value for still air; where the air movement is greater it may be estimated from the kata-thermometer readings. The "comfort zone" is likely to vary somewhat with the previous history of the individual and that indicated in figure 1 can only be expected to hold for subjects used to a climate of the American type. The conditions vary with the amount of clothing. Clothing interferes much more with the interchange of heat by conduction and convection than by evaporation (see also

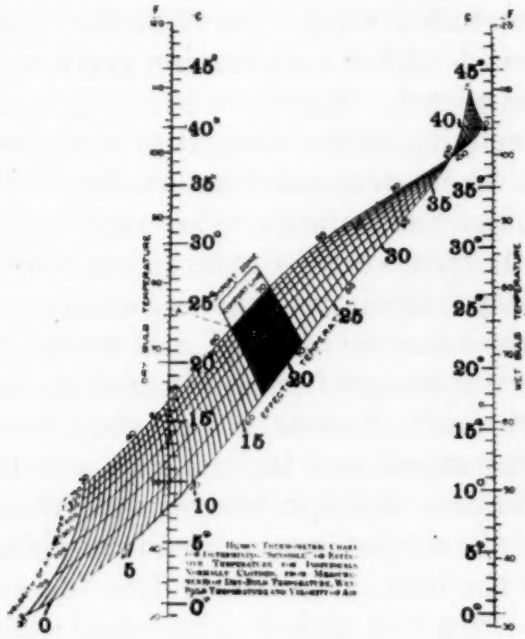

Fig. 1

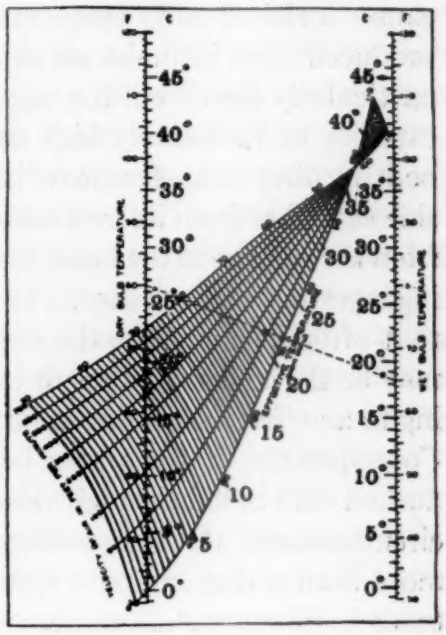

Fig. 2

Fig. 1. Effective temperature scale for subjects fully clothed, as given by Yagloglou and Miller, 1925.

Fig. 2. Effective temperature scale for subjects stripped to the waist, as given by Houghton and Yagloglou, 1923.

Vernon, 1923), so that at dry bulb temperatures above the body temperature clothes may be an advantage, interfering less with heat loss than with heat absorption. The values obtained with the subject stripped to the waist probably represent more closely the effect on exposed skin surfaces.

Unfortunately it has been necessary in the experiments of Yagloglou and his co-workers to return to measurement of cooling powers in terms of a temperature scale, although expression of heat loss in calories would perhaps have helped to clarify thought. An individual under basal

conditions produces heat approximately at the rate of 1/1000 of a small calorie per square centimeter surface per second, so that any condition which makes it hard for him to lose this amount of heat and calls forth physiological adaptations, which are often exhausting, should be considered warmth; if, however, he is doing work and producing more heat, conditions with a greater cooling power must be considered warmth. While the Pittsburgh group have recognised these principles, they have not yet been able to express effective temperature in calorific values.

Hot water (e.g., a hot bath) only a few degrees above body temperature causes a rise of body temperature which is much more rapid than that produced even in moist air at a much higher temperature, and this is particularly the case if the water be stirred. This is due to its high heat capacity and relatively high conductivity, which allows it to warm the body by direct transference of heat. The body thus obtains heat from outside as well as from its own metabolism, and is unable to lose heat except from the surface of the head, from the respiratory passages and by warming ingested food or water. The body temperature may rise so rapidly, that after a short time the conditions may be reversed, and the water may be the colder; as a result the water changes from an effective warming to an effective cooling medium owing to its same physical properties. Consequently, if the water be kept stirred and the subject's skin be flushed with blood through vasodilatation, as it commonly is under these circumstances, the temperature of the subject is prevented from rising more than a degree above that of the bath. While, therefore, hot air tends to give a rectal temperature rising with an increasing speed, a hot bath on the other hand gives the quickest rise at first followed by a slower rate until eventually, if the bath is not too warm, an equilibrium is reached at a new level. Sweating may be profuse in a bath, and may continue even when a uniform temperature is maintained at a high level, but, since evaporation is prevented, it produces desiccation of the subject without lowering the body temperature.

Radiation will only receive brief mention here. Light radiation will be dealt with in another review in this journal (Laurens, 1928). Loewy and Dorno (1925) found that infra-red radiation gave temperature changes in the tissues similar to those seen with hot applications acting through conduction, except that the effect was greater on the superficial than on the deeper layers when this form of radiant energy was used. On the other hand, the same workers found that if sunlight radiation was used in a cold room, the temperature of the deeper layers of the skin was raised as much as, or more than, that of those more superficially

situated. In this case the radiant energy penetrated well, and the temperature of an exposed limb might be raised to a higher general level without the temperature gradient through the tissues being reduced.

Muscular exercise increases the heat production in the muscles and the blood distributes it throughout the body; gradients are, however, probably considerably altered.

ACTUAL TEMPERATURES EXISTING IN THE BODY AND THEIR VARIATIONS. Changes in external temperature produce their effects, as might be expected, by causing variations in body temperature, in spite of the regulatory mechanisms of warm-blooded animals. While it is true that this class of animals succeeds in maintaining an internal temperature which is remarkably uniform, and which shows only slight variations, even when the environment is considerably altered, yet the mean temperature of the body as a whole is not really constant. The conception that a man has an internal mass of tissue maintained at about 37° surrounded by a thin layer with a steep temperature gradient, such that the surface temperature is about 33°, is common but inaccurate. The surrounding layer of variable temperature is not thin nor is the gradient necessarily very steep; on the contrary, the temperature of the whole mass of the limbs at rest may be much below that of the rectum. Claude Bernard (1876) recognised this fact; he showed that even in the large arteries the temperature of the blood was not quite constant, but that it increased slightly in passing from the thoracic to the abdominal aorta, and then fell progressively beyond the origin of the renal arteries. These temperature changes he ascribed to the interchange of heat between the arteries and the veins lying near them, veins containing warm blood from the liver and intestines tending to raise, and those containing cool blood from the periphery, to lower the arterial temperature.

Thus he recognised that the venae comites played an important part in maintaining an even distribution of heat. While the difference in temperature between the femoral artery and vein in a dog might only be 0.25°C. with a room temperature of 16°, yet on a very cold day he found that it might amount to 3 or 4°C., and he estimated that under such conditions the temperature of the blood must fall considerably while still within the smaller arteries, owing to the transference of heat between the arteries and the cool veins near them. Arterial blood need not therefore be delivered to the skin, or even to the other tissues, at temperatures as high as those existing in the heart or rectum. In calorimetric experiments on blood flow G. N. Stewart (1911) obtained good

evidence that the arterial blood entered the hand under ordinary room conditions a full 0.5°C. below the rectal temperature, since, if the water in his calorimeters was above this value, heat was given to the hand rather than withdrawn from it. That the veins may carry back very cold blood from the extremities is indicated by experiments of Lewis and Love (1926), who found that the temperatures as low as 16° might be recorded on the skin immediately over the veins, if the hands were exposed to cold water.

Liebermeister (1875) calculated from experiments with warm baths on himself (weight 50.1 kilo) the amount of heat necessary to raise his whole body to a temperature of 36°. He estimated this under normal conditions to be approximately 25 large calories, and showed that it might be double that amount if the surface had previously lost heat through exposure for a few minutes to a cold bath; such a large amount of heat implies a cooled superficial layer of considerable depth. Data in support of such a hypothesis have been obtained by many workers, and some accurate observations were even made with thermocouples by Becquerel and Breschet in 1835. Very convincing evidence of this cooled area has been advanced by Zondek (1919, 1920) who introduced through a trocar a mercury thermometer of fine dimensions. Zondek's thermometer is clumsy and must have a relatively high heat conductivity, making it a poor method for measurements of tissue temperatures near the surface, but it should have little or no error when inserted to considerable depths. In patients under the conditions of a hospital ward he often obtained readings at the depth of the linea aspera of the femur more than 1°C. below the rectal temperature. The fall of temperature between the fascia and the skin was greater the fatter the subject, and it could be much increased in all subjects, if the environmental temperature was lowered. Zondek showed by direct measurement that this cooling effect might extend through the fat into the more superficial layers of the muscles, particularly in the thinner subjects. That the temperature variations produced by external conditions penetrate to considerable depths has also been demonstrated in animals for the head by Leyton and Sherrington (1917), for other parts by Macleod and his co-workers (1920, 1921). The former noted that a bag or hot water bottle applied to the skull might readily change the intradural temperature through a range of 5 or 6°C.; the latter found that an applicator 12° to 16°C. above the body temperature might cause a rise of as much as 0.3° within the intestine at a depth of 5.4 cm., and that one only 4.7° above body temperature applied to the head, could cause a rise of over 0.5°C. at a depth of 17 mm. within the brain.

Lefèvre (1911) has also published curves representing the temperature gradients in the subcutaneouus tissue in man and demonstrating the existence of a thick layer of cooled tissue, which varies considerably both in depth and temperature according to the environmental conditions. Unfortunately the actual data on which his curves are based are not given in detail, and additional figures have been obtained to determine the exact character of these curves. Some of these have been reproduced, others are in course of publication (Bazett and McGlone, 1926 a, b, 1927) and all demonstrate conclusively the existence of a large and variable cooled area. The values obtained are in close agreement with Liebermeister's data. Thus in the curve (1926a) representing the temperature gradient in the thigh on H. C. B., when sitting, clothed only in a bathing suit, in a room at 21.7°C., the temperature recorded in the sartorius muscle was only 35.3°C. at a depth of 19.2 mm. Extra-polating the curve obtained, and supposing that the gradient observed in the thigh was one not far removed from the mean gradient of the whole body (though actual figures obtained shortly after this demonstrated that the gradient in the forearm was of a somewhat different character), it is possible to make an approximate calculation of the number of calories that would be required to raise the whole body of this subject to 36°C. The value so obtained is 39 large calories for a subject of 65 kilos weight, when feeling somewhat cold. The value therefore is quite comparable to that estimated by Liebermeister for a smaller subject. That this cooled area may vary considerably under different environmental conditions is also indicated by the considerable changes that may occur in surface temperatures, changes long known and which are reviewed in a recent article by Cobet (1926). Benedict and his coworkers (1919, 1925) have in particular recently made careful studies of these changes, and have pointed out that since temperatures much below 30° may be recorded on the limbs after exposure to cold, these temperature changes must penetrate to a considerable depth. In support of this assumption they observed that these low surface temperatures could not be much raised, even if the surfaces were protected from exposure to air and placed in conditions similar to those of the deeper tissues by the crossing of the arms or legs. Figures comparing the skin temperature with the cooling power estimated by the kata-thermometer are given by Loewy and Dorno (1925), and as estimated by the "effective temperature" scale by McConnell and Yagloglou (1924). The agreement is fair in both cases, but the "effective temperature" scale appears the better index.

In small animals the extension of the cooled layers may be relatively so great as to result in an instability of internal temperature. It is possible that such factors play a part in the relative instability of the rectal temperature in children. Yoshinaga (1925) has demonstrated in the mouse that the rectal temperature may readily vary more than 4°C. under varying climatic conditions. He showed also that upon exposure to warmth the gradients are diminished and the temperatures of the subcutaneous tissue and the rectum approach one another. In the mouse exposure to a warm room may cause an initial fall of rectal temperature. A similar initial fall on exposure to warmth has been described in dogs and rabbits by Obernier (cited by Pembrey, 1898).

Even in man climatic conditions may affect not only the surface but the deep body temperature, for the rectal temperature is in general somewhat higher the warmer the environment. Evidence of this has been recently obtained by Lee and Edwards (1914) who found that the rectal temperature of a number of subjects averaged 36.72° after 7 hours in a room temperature of 20° (50 per cent humidity), 36.99° under similar conditions at 23.9°, and 37.41° when the temperature was 30° (humidity 80 per cent). Similar observations by Neuhauss and others which are cited by Pembrey (1898), indicate that the rectal temperature is definitely slightly higher in tropical climates than in the temperate zones.

During the period of adaptation a reverse relationship may be seen; thus Liebermeister (1875) showed that exposure to cold usually causes an initial rise of rectal temperature, though it is less certain whether in man an initial fall of rectal temperature commonly follows an exposure to warm conditions. Usually the exposure to warmth has been extreme and such an initial fall has not been noted. Liebermeister (1875, p. 105) demonstrated that if a subject were exposed to cold (naked in room of 20 to 21°C. accompanied by wetting with a damp towel for 23 and 53 minutes) the rectal temperature might rise slightly during such an exposure, but that a fall of temperature occurred as soon as the subject was again covered with a blanket (the rectal temperature fell 0.32° in 25 minutes and 0.45° in 32 minutes in these two experiments). Following exposure to cold, therefore, the subject's deep temperature may fall when he is again exposed to warmer conditions, and this must be a common occurrence in normal life. Such a reaction has also long been recognized in the literature in the form of the after reaction (fall of rectal temperature in a warm room) following a cool bath (Strasser, 1923). A slight initial fall is also sometimes demonstrated on slow

warming in a bath as in some of the experiments detailed by Strasser (1923).

That a rise of rectal temperature ultimately occurs when a person is subjected to really high temperatures is not surprising, and is evidenced in numerous papers. The work of the Pittsburgh group shows that the temperature rise in a half-clothed subject commences when he is exposed to an "effective temperature" above 30°C. (basic scale), and that the rate of rise of temperature may be demonstrated to be related closely to the "effective temperature" (Houghton and Yagloglou, 1923a). Rectal temperatures as high as 40.2° (Graham and Poulton, 1912) have been recorded from exposure to warm air, and in hot baths as high as 40.8° (Haggard, 1920) and 40.7° (Bälz, 1893).

When the individual is exposed to new conditions, new temperature gradients are set up in the tissues and readjustments of heat distribution throughout the body follow. Some data are available as to the course of these changes in the limbs with local application of heat, and also for more general changes of environmental conditions, and, though the data are as yet scanty, they must receive some consideration. Loewy and Dorno (1925) following Zondek (1920) have reported observations on the penetration of temperature changes as the result of local hot and cold applications. Cold applications produced a rapid fall of surface temperature and the temperature of the tissues some 2 or 3 cm. deep fell at first quickly, then slowly, showing ultimately quite a profound fall. In one experiment on the thigh the surface temperature fell from 31.6° to 22.1° in one and a quarter hours and in the same time the temperature at a depth of 2 to 3 cm. fell from 35.2° to 29.5°. The temperature gradients were therefore steepened. On removal of the cold application the original surface temperature was only regained slowly showing a rise of some 3° in the first 8 minutes, but the deep tissues showed a much slower change requiring 50 minutes to rise 5.7° and their temperature was considerably below normal at the end of 90 minutes, some time after the original surface temperature had been regained. Though the temperature gradients were at first steepened, they were during recovery diminished to a subnormal level. The prolonged diminution in the deep temperature might be accounted for, if part of the warming of the superficial tissues was attained at the expense of the deeper tissues by direct conduction of heat. With warm applications the temperature gradients were at first reduced and then reversed, but at a depth the effect was less and the change progressed more slowly than with cold of the same intensity; the rise of deep temperature was

initially fairly rapid, then very slow. On removal of the warm applicator the surface temperature fell at first rapidly then very slowly. Thus in one experiment the surface of the thigh rose from 30.2° to 42.4° in 5 to 6 minutes, but in the following 15 minutes rose less than 1°C; the temperature at 2.5 cm. depth rose from 34° to 38.2° in 28 minutes (one-half of this rise in first 5 minutes). On removal of the applicator one-half of the total gain in surface temperature was lost within one to two minutes, then the loss was much slower, so that even half an hour later the surface was still 2° above its normal value; the deep temperature recovered very slowly falling only 1° in 4 minutes and in 38 minutes it was still 1.3° above its normal value. On recovery therefore the temperature gradients were quickly reëstablished although they may have been temporarily exaggerated, and then returned to a normal or subnormal value. The authors attributed the slighter effect on the deep tissues to the wider distribution of the heat by the increased blood stream flowing in dilated vessels; the later maintenance of a raised surface temperature received a similar explanation.

These local changes probably exemplify what may occur in the body as a whole. The gradients in the skin, subcutaneous tissue and muscle layers in the limbs can be shown by direct measurement to be much less steep under warm conditions than those found when the air is colder (Lefèvre, 1911; Zondek, 1920; Bazett and McGlone, 1927). Though no observations at present exist on the change of the one gradient into the other on general exposure of the whole body to warmth, yet a few isolated figures have been obtained on the alterations of these gradients in the return to a normal condition after cooling (Bazett and McGlone, 1926, 1927) and the direction of change both of temperature and of vasomotor tone are probably similar in the two cases.

In the experiments to which reference is made, the cooling resulted from some 9 or more minutes' swimming in water at 20° to 21.5°C., after which a fall in deep body temperature of over 0.5°C. was commonly observed. At the same time the surface temperature, as well as the temperature of the muscles of the forearm, was very much lowered; on the other hand, if shivering was in evidence the temperature within the sartorius muscle in the middle third of the thigh might be slightly raised (0.3°C.). The gradients were everywhere immensely steepened. As the subject became warmer the surface temperature rose considerably while the rectal temperature rose only slightly, remained unchanged or even fell slightly, and the muscle temperatures consistently fell, so that the gradients were much reduced and might return to a normal

or even to a subnormal type, before the normal temperature level was regained. The return to a normal gradient even at an abnormally low level was interpreted by the subject as "comfortable," and on the two occasions when an abnormally flattened gradient in the peripheral subcutaneous tissue was set up with a surface temperature about normal, but a rectal temperature still much below normal, the subject experienced a "warm glow."

The mechanisms involved are probably as follows. The heat lost from the surface in the absence of sweating is mainly dependent on the difference in temperature between it and the air, and the transference of heat through the subcutaneous tissue and skin is proportional to the temperature gradient, if the heat conductivity of these tissues is maintained constant. If, however, the heat conductivity of the skin be increased, as for instance as the result of vasodilatation, then the same amount of heat may be transmitted at a lower gradient. After the cold bathing, the heat lost from the surface would be much decreased as the result of the fall in surface temperature, but the heat transmitted across the subcutaneous tissue might be quite high as a result of the steep gradient, even in spite of probable vasoconstriction. Heat would therefore be transmitted to the surface over and above that lost from the surface, which would be warmed at the expense of the deeper lying tissues, which in turn would be cooled by the venous blood. In this way the normal gradient would be reëstablished, or even a subnormal gradient be created, if the surface temperature and consequent heat loss still remained subnormal. Following the bath, when such a subnormal gradient has been set up, the temperature of the body as a whole appears to rise gradually, probably until the mean value of the surface temperatures reaches its normal level relative to the environment and the heat production and heat loss again balance, when normal gradients are reëstablished. Such a series of events must clearly be modified by vasomotor changes, but in general the above hypothesis would account for the temperature changes actually observed in recovery from both local and general exposure to cold.

If now a normal subject be exposed to a warmer environment a similar chain of events is to be anticipated; the heat loss from the surface will be diminished and consequently as before the surface temperature will rise, and this rise of temperature will be related to the effective temperature (McConnell and Yagloglou, 1924). If no vasomotor adjustment were possible the temperature of the body as a whole would also rise so that the requisite gradient might be established; but the

warmth appears to lead to vasomotor adjustments which increase the heat conductivity of the subcutaneous tissue, allowing the heat to be conducted through it with a lower gradient, and the surface temperature to be raised to the necessary extent without much alteration of the internal temperature. The initial fall of body temperature on exposure to warmth described in dogs, rabbits and mice, and occurring sometimes in man, would therefore be explained as due to a vasomotor dilatation induced in response to the warmth, producing a considerable flattening of the temperature gradients, and rise in surface temperature, which is temporarily produced at the expense of the deeper tissues.

In general therefore gradients are minimized by exposure to warmth and recovery from exposure to cold and exaggerated by exposure to cold or recovery from exposure to warmth; vasodilatation tends to minimize and vasoconstriction to exaggerate them (Taylor, 1923). In recovery from exposure to cold the physical factors can perhaps account for the changes observed, in recovery from warmth vasomotor changes may be more important, but the course of events needs further investigation. No doubt in both cases changes in temperature of one part of the body may occur at the expense of another, resulting in considerable variations in the mean temperature of the body without this being demonstrable by measurement of the rectal temperature, which may indeed show temporarily an opposite change to that of the body as a whole.

With heavy muscular exercise a rise of rectal temperature is also common, and values as high as 39.4° (MacKeith and his co-workers) in the English climate and as high as 40° in America (MacKenzie, 1924) are reported. It has however been demonstrated that this rise of rectal temperature may not be detectable for some minutes, even though the exercise be undertaken in a warm room, and sweating may commence before any such rise is demonstrable (Berner and co-workers, 1926). Under such circumstances however the axillary temperature shows a rapid rise so that the delay in the rise of rectal temperature may be caused either by a vascular dilatation which leads to a redistribution of heat, or by lag due to the heat capacity of the rectum. But, while the application of external heat tends to obliterate or even reverse the temperature gradients in the tissues, exercise must exaggerate them at any rate locally. The temperature of parts of the surface may perhaps show a rapid rise (judging from the axillary temperature), and since the skin receives its blood supply from arterial branches which pass through the muscles and pierce the deep fascia (Spalteholz, 1893),

its temperature in the active area is likely to be more rapidly affected by muscular activity than that of the rectum. The temperature gradient within the active limb is apt to be steepened, but it may be reduced in other parts of the body to a subnormal value through vasodilatation.

The fact that the direction of temperature change is commonly not the same in different parts of the body makes it questionable as to what should be considered body temperature or even blood temperature. Under ordinary conditions when the body is nearly in thermal equilibrium, the rectal temperature is considered equivalent to that of the blood in the aorta, and animal experiments would appear to justify this assumption. The temperature of the urine while being voided is close to that of the rectum, and is according to Pembrey (1898) about 0.2° lower, though in my own experiments where the two observations have been made simultaneously the difference has rarely been as great as 0.1°. The temperatures of the mouth and axilla are lower, as might be expected since they are supplied by arteries which are in close proximity to veins containing cooled blood. When the temperature is rapidly changing the above assumptions need no longer be true; thus the lag in the temperature change of a large volume of urine in the bladder is very great; if the bladder be not emptied before a swim in cold water, urine temperatures 0.5° or more above the rectal temperature are readily obtained. Such a lag is not surprising in view of the small blood supply of the mucous membrane in relation to the high heat capacity of the urine, so that the urine temperature is much affected by the mean value of the temperature of the kidney during the period of urine formation. The contents of the rectum are usually of smaller volume, but cannot be expected to follow blood temperature changes without lag. The axilla has perhaps in proportion to its heat capacity a higher blood supply, and the mouth one greater yet, so that any such lag may be expected to be least in the mouth, greatest in the rectum, and variable in the urine according to the rate of urine formation. Unfortunately the mouth temperature is apt to be affected also by external conditions, as through inspiration of air, and the temperature of its blood supply may also be affected by that of the blood in the veins of the neck.

On exposure to warm air, McConnell and Houghton (1923) and Adolph (1924) have reported instances where the rate of rise of mouth temperature was more rapid than that of the rectum, and where the mouth temperature might be consistently above that of the rectum for a long period of time. Such results may be due mainly to the warming of the mouth cavity directly during respiration or to the warming of the blood

in the external carotid artery by its close contact to the jugular vein, but one factor may be lag in the rectal changes. In the absence of thermal equilibrium therefore the rectal temperature may be taken as evidence of the direction of any change in the temperature of the aortic blood, but not of its temperature level.

CHEMICAL CHANGES DUE TO VARIATIONS IN TEMPERATURE. Variations in temperature have been demonstrated to affect the velocities at which many bodily functions are performed to an extent which is similar to that produced by temperature changes on the velocities of chemical reactions, and the similarity has been taken as evidence that the velocity of such functions is determined by some fundamental chemical reaction, even though similar high coefficients may be observed in some physical processes (Gasser and Hill, 1924). The isolated heart of many animals, if not of all, is affected by temperature changes (within the range at which the tissues function) to an extent such that the rate is increased 2 to 3.5 times if the temperature be raised 10°C. (Kanitz, 1915; Snyder, 1912, 1913) and the refractory period of muscle (Bazett, 1908) and nerve (Adrian, 1921) as well as the chronaxie of muscle (Filon, 1911) have all been shown to be affected similarly by temperature. The factor by which the velocity at any given temperature must be multiplied to give the velocity at a temperature 10° higher is commonly written as Q_{10}. It may be calculated by the following formula

$$\log Q_{10} = \frac{\log K_1 - \log K_2}{t_1 - t_2} \times 10$$

where K_1 is the velocity at the higher, K_2 that at the lower temperature, and $t_1 - t_2$ indicates the difference in temperature.

Crozier has attempted to analyze the effect of temperature on numerous biological processes both in plants and animals (mostly cold-blooded) in terms of Arrhenius' equations for the effect of temperature on chemical reactions (Arrhenius, 1915), and comparisons are then made in terms of variations in the constant μ. His results are reviewed in papers recently published (Crozier, 1924, 1926; Crozier and Stier, 1926). He assumes that the biological processes are the result of a series of linked chemical reactions, and that at certain critical temperatures the limiting chemical reaction may be changed. His calculations are not always convincing, but none the less he has obtained good evidence that the velocities of many complex biological processes, including reflex activities, often appear to be dependent on relatively simple chemical processes such as those involving H and OH ions. In man, therefore,

one must expect variations in the rate of chemical activity in the skin, subcutaneous tissue and to some extent muscle as the direct result of local physical changes in temperature and apart altogether from reflex action. Since the metabolism of the skin and fat is probably low at any time, this need not be important, but it is probably not negligible in the case of muscles, nerves and nerve endings, and presumably alters not only their excitability but also their refractory period and chronaxie. The relationship of any such changes to the stimulation of end organs (including those sensitive to temperature) is not known, but may be important, particularly since the recent work of Adrian and Zotterman (1926) suggests that the end organs have a long refractory period, that the gradation of a sensation depends on the frequency of the impulses transmitted to the nerve, and that this is dependent on the refractory period.

In addition to the effect on velocity of chemical changes, alterations in temperature may also affect considerably the equilibrium point of reversible chemical reactions. It has long been known that this is the case in the dissociation of oxy-hemoglobin (Barcroft, 1914; Brown and Hill, 1923; Stadie and Martin, 1924).

Recent work has also demonstrated profound effects on the acid-base equilibrium (Stadie and Martin, 1924; Austin and Cullen, 1925; Stadie, Austin and Robinson, 1925). It has been demonstrated by these workers that, in addition to decreased solubility of CO_2 and an increased dissociation of electrolytes, the base-binding capacities of blood proteins are much increased by a rise of temperature, so that they combine with more base, raising the tension of CO_2 and changing the pH to the acid side unless the CO_2 is able to escape. Their curves demonstrate that if a normal blood had a pH value of 7.37, pOH of 6.27 and a CO_2 tension of 40 mm. Hg when in the lungs at a temperature of 37°, the pH value would be changed to 7.51, the pOH to 6.30, and the CO_2 tension reduced to 27 mm. in the arterioles of the skin, if the blood temperatures were reduced to 30° without any opportunity for additional CO_2 to be taken up. Such a condition therefore probably represents the composition of the blood being delivered to the skin under conditions which are not uncommon in a cool room. If, on the other hand, the skin be exposed to warmth, so that warm blood is passing back along the venae comites a fall of temperature of the arterial blood may be prevented, or, under extreme conditions, a rise of temperature may be induced. In the former case the blood would be delivered in a similar condition to that existing in the lungs, in the latter the blood would be even more acid and

the CO_2 tension higher (e.g., a pH of 7.28, a pOH of 6.25 and a CO_2 tension of 51 mm. might be expected at 41°, again supposing that no exchange of CO_2 had taken place). If, however, CO_2 is able to escape and the tension of CO_2 rather than the total amount be kept constant, then cooling causes a considerable increase in the pOH value with little change in pH, i.e., an increased acidity through a diminution in OH ions with little change in H ion concentration. These changes are great and if they occur in vivo, as in vitro, they must be of extreme importance. A few preliminary experiments have been made by McGlone and the writer to determine whether this is the case. Decerebrate cats were injected intravenously with 30 cc. of a 2 per cent neutral solution of phenolsulphonephthalein, which caused an immediate intense red colour in the skin but which did not appear to be associated with any other marked change in the animal. The dye is one recommended for tissue pH estimations by Rous (1925). The two fore-limbs of the cats were partially shaved and were immersed in two water baths, one at 5° to 10° and the other at about 45°. The skin of the cooled limb soon assumed a bright reddish-purple color, while the warmed limb assumed a slightly orange-red tint. Only a rough estimate of the difference in pH existing between the two limbs could be made, but it was judged as 0.2 or more. No color difference was detectable, unless the subcutaneous temperature of the two limbs differed by more than 10°.

It would appear, therefore, that the changes observed in vitro occur also in vivo, if temperature changes are considerable, and that the amount of CO_2 contained in the blood is more nearly constant than its tension. But the changes observed are less than those observed in vitro, so that vasomotor changes may be affecting a partial compensation. Dautrebande (1924) and Goldschmidt and Light (1925b) have investigated the venous blood of the forearm with exposure to cold. Both found the blood to have a higher oxygen content, so that changes in pH might in part be due to a metabolic rate lowered by the fall in temperature; Dautrebande also found a lowering of the $BHCO_3$ level, and noted a change to the acid side (pH 7.32 changing to 7.19), if the ratio of free and combined CO_2 was measured, and the pH estimated at the normal rectal temperature level. The alteration in the local circulation rate seems, therefore, to render the blood more acid, if the direct effect of the temperature change be neglected, but the opposite change produced by the actual alteration in temperature overbalances this and causes an increased alkalinity. The occurrence of such changes would also result in modifications of osmotic pressure (Austin, Sunderman and Camack, 1927).

It is probable that changes in temperature may also similarly alter the acid-base equilibrium in the tissues, though nothing is known of the magnitude, nor even of the direction, of such changes; in addition, it is unlikely that acid-base equilibria are the only ones modified by temperature.

TEMPERATURE SENSATIONS. The sensations of temperature induced by the physical changes are of importance since they are associated with reflex responses. These sensations arise from the end organs which lie beneath the spots of the skin which are sensitive to cold and warmth (Blix, 1884; Goldscheider, 1885; Donaldson, 1885), and possibly also from other end organs. A review of the older literature has been given by v. Frey (1910).

The *cold spots* can be stimulated by application of a metal point some 8° to 9° above or below the surface temperature, if the applicator has a heat capacity adequate to produce the necessary temperature change in the tissues. Both warm and cold stimuli can produce a sensation of cold. If, however, the warm stimulus affects a warm spot as well as a cold, the combined sensation is interpreted as "hot" (Altrutz, 1900; Hacker, 1913). The cold spots must be considered identical with Krause's end bulbs, at least in the conjunctiva, for recent work from v. Frey's laboratory demonstrates that the distribution of these end organs and that of the cold spots are very similar, and such end organs have even been intravitally stained and identified just beneath spots sensitive to cold (Strughold and Karbe, 1925a, b; Strughold, 1925). v. Frey noted in 1895 that the cold spots were commonly situated close to blood vessels and Strughold notes that this association may be readily demonstrated in the conjunctiva. The cold spots on the prepuce are also very definitely related to blood vessels, being situated along the course of small venules (unpublished observations by the writer). The latent period of the cold sensation in the forearm varies with the strength of the stimulus (v. Frey, 1910), but, if the cold stimulus be below the surface temperature and in intensity close to the threshold value, a latent period of about 0.4 to 0.5 second was obtained in some unpublished observations by McGlone and the writer on the rate of penetration of temperature changes. The reaction time for touch is about 0.14. One may therefore estimate that the temperature changes with the stimuli used take about 0.3 second to penetrate to the depth of the end organ.

The *warm spots* are fewer in number, give more diffuse sensations and are less easily identified than the cold spots; they are less readily

affected by local anesthetics (Hacker, 1914; Strughold, 1923; Rein, 1924), though this has been denied by Goldscheider and Hahn (1924) on somewhat unconvincing evidence. These end organs are, therefore, thought to be more deeply situated than those of the cold spots, but they have a lower threshold and may be readily stimulated by temperatures 6° to 8° above the surface temperature. A cause of much controversy (see Rein, 1924) has been the paradoxical sensations of warmth in response to cold stimuli only a few degrees below the surface temperature which have also been described; they are difficult to elicit and have been attributed by some to mechanical stimulation of end organs. Rein himself described a doubtful paradoxical sensation of warmth from cold stimuli applied to inflamed skin, and McGlone and the writer have also found paradoxical sensations of warmth to be less rare, when the skin has an inflammatory hyperemia (unpublished observations). The reaction time to warm stimuli slightly above threshold intensity has been in our experiments 1.1 to 1.3 seconds (with weaker stimuli v. Frey (1910) reported latencies as long as 3 seconds). From a comparison with touch sensations, these results suggest that the end organs for heat exist at a depth to which these temperature changes penetrate in about one second.

Some indication of the actual *depth of the end organs* for cold and warmth is supplied by data we have obtained as to the rate of penetration of the temperature changes in these same experiments. The time of penetration is approximately a linear function of the depth, and occurs at the rate of about 0.5 mm. per second for cold and 0.75 mm. per second for warmth. The linear relationship seems to indicate that the main transmission has been along the blood stream, and the quicker penetration of warmth would depend on a vasodilatation and would correspond with the more gradual change in temperature in the deeper tissues noted by Loewy and Dorno (1925).[1] If this explanation is correct the vascular dilatation to warmth appears to be induced rapidly, probably before sensations of warmth can be recorded. The values obtained for reaction time suggest that the end organs in the forearm lie at a depth of less than $\frac{1}{2}$ mm. for cold and between $\frac{3}{4}$ to 1 mm. for warmth.

Goldscheider (1898) supposed on theoretical grounds that the maximum rate of temperature alteration should be reached in times proportional to the square of the depth. This may be the case for the most

[1] The decreased time for penetration would depend on the change in thermal conductivity, the slower rate of change on the altered thermal capacity.

superficial layers devoid of a blood stream, but is apparently untrue at greater depths, though it is somewhat difficult to determine accurately the time of the greatest rate of temperature alteration. Sometimes the initial temperature changes recorded are opposite in direction to those anticipated, showing that the results are complicated by vasomotor changes as well as by conduction along the blood stream (Bazett and McGlone, 1926b).

The experiments of Pütter (1922) would indicate that the end organs concerned in estimating small temperature differences must also be superficially situated, and may therefore be identical with those already described. He found that discrimination between two surfaces, whose temperatures only differed by 0.5° or 0.7°, could be attained within one to two seconds or less in the palm of the hand, so that, according to the data already quoted, the end organs could not be more than 1.0 or 1.5 mm. deep. He himself calculated the depth to be only 0.1 mm. on theoretical grounds, but such calculations were unwarranted, as his premises were obviously unsound. Evidence of their fallacies is supplied by his calculation that 130 seconds would be required for the maximum velocity of temperature alteration to be attained at 1 mm. depth, when actual observed values are only about 1/100 of this.

The *general sensations* of bodily warmth experienced on a hot day do not appear subjectively to be identical with those felt on entering a warm bath, nor does the sensation of cold experienced after some 20 or 30 minutes swimming in cold water seem identical with the sensation experienced on entering the water. It is therefore possible that other end organs for temperature exist in a deeper situation, and that they give rise to proprioceptive rather than exteroceptive sensations of temperature. However, if such end organs exist, they have not as yet been demonstrated. Some evidence of temperature receptors in the muscles themselves has been brought forward by Freund and Janssen (1923) as the result of investigations into the oxygen consumption of muscles at different temperatures, and the effect of nerve section upon it; Mansfield (1910) also has supposed sensations of temperature to arise in the pericardium. The data in both cases are complicated, so that the evidence for such sensory endings is very indirect, and the data obtained by Mansfield are open to serious criticism (Kirsch, 1923).

Head and Rivers (1908) described an additional type of temperature sensation in the skin, one with a lower threshold which distinguished differences of less than 1°, but which was only experienced if the applicator had a relatively large surface. The separate identity of these

so-called epicritic sensations has not been confirmed by others (Trotter and Davies, 1909; Boring, 1916), and the phenomena described can perhaps be accounted for on other grounds. If a weak stimulus be applied over a wide area, the actual temperature change beneath the skin may equal that of a greater stimulus applied to a small area, and this will be especially true if the end organs are situated close to venules and so are affected by venous blood temperature. It is also likely that subliminal stimuli to end organs may produce impulses which ascend the nerve trunks but become blocked in the central nervous system, but that, if a number of different end organs are so stimulated, summation of these subliminal impulses may occur in the nervous system. The temperature sensations which may be elicited from the glans penis are very different from those of the prepuce, as Head and Rivers pointed out, but the difference might be due to the number of sensitive spots present or even to differences in the arrangement of blood vessels. Waterson's criticism (1923) of the localization of temperature sensations at definite spots has neglected both physical and physiological principles of heat conduction.

The mechanism of stimulation of the end organs is uncertain. Their refractory period, and consequently the frequency of the impulses induced, is likely to be affected by the absolute temperature, but the sensations produced seem only slightly dependent on the actual temperature level. Weber (1846) supposed a cold sensation to be induced by a fall of temperature and one of warmth by a rise; this view is still possible, in spite of paradoxical sensations, since the temperature changes themselves may initially be paradoxical in direction (Bazett and McGlone, 1926) probably as the result of vasomotor changes, though such changes are short lived and the sensations may more probably be taken as evidence that the end organs are sensitive to temperature changes regardless of their direction. Ebbecke (1917) supposed the stimulus to be the production of abnormal spatial temperature gradients in the region of the end organs, and the sensation experienced to be an integration by the central nervous system of the sensory impulses so induced in the two types of end organ; on his theory one important element is the temperature of the blood supplying the area. Evidence of the existence of sensory integration of the type he supposed had already been advanced by Head and Rivers (1908) in their experiments on the sensitivity of the glans penis. Spalteholz (1893) described the separation of the arterial and venous plexuses in the superficial layers of the dermis and the depths suggested earlier in this paper for the end

organs of cold and warmth would be close to those of his 1st and 2nd venous plexuses. It is therefore conceivable that the end organs are related to these blood vessel systems and that they react either to alterations in venous temperature or to changes in the temperature gradients between arterioles and venules. The difficulty in the production of paradoxical sensations of warmth, diminished in the presence of inflammation, might be due to the induction by cold of a vasoconstriction which prevented the necessary rapidity of temperature alteration in the deeper venous plexus, and though the phenomena of temperature adaptation may be in part nervous in origin, alterations in the temperature of the arterial blood in the neighborhood of the end organs appears also to be an important factor, for v. Frey (1910) noted that the power of adaptation is greater, the more peripheral the position of the skin investigated. The theories of Ebbecke certainly deserve serious consideration, in spite of the criticisms advanced by Goldscheider and Hahn (1924).

METABOLISM. *On exposure to cold the total metabolism* is increased and this change constitutes the chemical method of heat regulation. There has been considerable dispute as to whether the increase is induced solely through muscular movement, including shivering, or whether other mechanisms are brought into play. Evidence has recently been brought forward by Hartman and Hartman (1925) as well as by Cannon and his co-workers (1927) of an influence of the adrenals on metabolism in response to cold through an increased output of adrenalin. The latter critically review the earlier researches on the relation of shivering to the increase in metabolism (see also Hill, 1919; Hill and Campbell, 1923) and adduce evidence that increases of metabolism may occur without shivering, and that under such circumstances an increased output of adrenalin is demonstrable through its effect on the denervated heart. Evidence has also been advanced by Mansfield and v. Pap (1912) and by Schenk (1922) of a varied degree of thyroid activity in response to temperature changes; Mansfield and v. Pap found that the isolated mammalian heart consumed sugar more quickly when the blood serum of an animal exposed to cold was added to the perfusion fluid, and Schenk that the metabolism of an animal was increased, when it was injected with serum obtained from a cooled animal; in both cases the effects were said to disappear after thyroidectomy. Mills (1918) has also described structural variations in the thyroid according to the environmental temperature of the animal. Reflex shivering or other reflex increases in movement probably constitute the

chief methods by which the metabolism is increased in response to cold. Possibly variations in muscle tone also occur but present data suggest that muscle tone is not associated with any high degree of metabolism (Roaf, 1912; Bayliss, 1912; Barenne and Burger, 1924). Other mechanisms such as the chemical ones described no doubt exist, though nothing is known as to whether they exert a constant tonic activity, and, if so, whether such activity may be diminished in response to warmth.

On exposure to excessive warmth the total metabolism is also increased, and the increase in this case appears to be due to the actual rise of temperature of the tissues and the consequent greater velocity of their chemical changes. Du Bois (1921) has obtained numerous values in a calorimeter on the heat production of individuals with abnormally high temperatures as the result of an infection or of an experimental protein injection. In all these experiments, which have been concerned with a relatively chronic rise of temperature, he has found the metabolism to be increased to a degree to be anticipated on the above hypothesis, and he estimated the Q_{10} factor (theoretical ratio of the metabolic levels for a difference of 10°C. in rectal temperature, see p. 546) to be about 2.3. In strong contrast with such deductions are the values for increase in oxygen consumption obtained when the temperature alteration is produced more rapidly. Figures obtained by Landis and his co-workers (1925) give an increase as great as 50 per cent for a rise in rectal temperature of 2.1° and by Koehler (1923) one of 97 per cent for a rise in rectal temperature of 2.7°. These data if used to calculated hypothetical Q_{10} values would give figures of 6.9 and 12.1 respectively; in both of the above instances rapid changes of body temperature were produced by hot baths. Plaut and Wilbrand (1922) describe an experiment with hot air on a subject who was incapable of sweating, where there was an increase of 107 per cent in metabolism with a rise of rectal temperature of only 2.8° (Q_{10} 13.4). On exposure to high room temperatures, and when the rectal temperatures were rising, McConnell and Yagloglou (1925) found an increase in metabolism from 37.6 calories per meter per hour with a rectal temperature of 37.0° to 70 calories at a rectal temperature of 38.8° (Q_{10} 31.0). The discrepancy between these results obtained by numerous workers with relatively rapid temperature changes and those obtained by DuBois under more chronic conditions in fever is very definite and needs explanation. Two possible hypotheses may be mentioned. In the more acute experiments a very pronounced hyperpnea is commonly induced, and this hyperpnea must itself cause an increased metabolism which may be expected to be greater in

baths where the respiratory movements have also to cause water displacement. Liljestrand (1917) has determined the increased oxygen consumption associated with the work of the respiratory muscles. He found this oxygen consumption to be greater with voluntary hyperpnea accompanied by alkalosis, than when the increased respiration was induced by carbon dioxide. According to his data a ventilation of 20 to 30 liters per minute might be expected to increase the oxygen consumption about 100 cc. per minute if accompanied by an alkalosis, or some 30 cc. if accompanied by an acidosis. A certain amount of the increased oxygen consumption, in experiments where the body temperature rises abruptly, could then be ascribed to the actual work of the respiratory movements, or to an absorption of oxygen, in a manner as yet unexplained, in the adjustments of the organism to an alkaline condition. In the case described by Plaut and Wilbrand there was no alteration in the rate of respiration, nor was any marked change in the depth of these movements noted, yet the oxygen consumption was enormously increased, so that some other explanation seems indicated. In such experiments a serious error arises, if the change in the mean body temperature is estimated from the alteration in rectal temperature only; such an error might account for the discrepancy. If one considers the experiments of DuBois, where a condition of thermal equilibrium was approximated, the rise of rectal temperature was probably matched by a similar rise in surface temperature, so that the change in rectal temperature did represent approximately, even though inaccurately, the change in mean body temperature; in bath experiments, or in hot air, when thermal equilibrium is far from being established, the rectal temperature does not give even an approximate estimation of the change in mean body temperature, for the surface temperature, which was originally many degrees below that of the rectum may be raised considerably above it, the gradients may be reversed and the alteration in the mean body temperature may be many times that of the rectum. A similar explanation would also account for the slight increase in O_2 consumption noted by Landis even with a bath at neutral temperature. When these complicating factors are taken into consideration, there is no evidence against DuBois' hypothesis that the increase in metabolism with rise of temperature is simply that due to the temperature coefficients of the chemical changes concerned, nor any reason to suppose that the conditions of hyperthermia and fever are not similar in this respect.

On exposure to intermediate temperatures the total metabolism variations are more controversial. Basal metabolism standards are those obtained

when the subject is exposed to ideal temperature conditions, such that no metabolic effort is required to maintain body temperature, while the cooling power is adequate to prevent a rise of body temperature. The basal metabolism should therefore represent the lowest possible level with the complete absence of any chemical response to cold. But difficulties have arisen owing to inaccuracies in standardising environmental conditions, and McConnell and Yagloglou (1925) claim that under the ideal conditions, when determined according to their "effective temperature scale," the basal metabolic rate is somewhat below that given as the normal standard.

It has been pointed out that endocrine influences are concerned in the reaction to cold; modifications of the activity of such glands might therefore be anticipated as the result of chronic exposure to different climatic conditions. Whether these modifications occur is uncertain, but some workers have obtained evidence that the basal metabolism is diminished as the result of exposure to a warm climate (Almeida, 1920, 1924; Knipping, 1923). Knipping finds that the basal metabolism is raised during the first week of exposure to a tropical climate, but that it then sinks below the ordinary accepted standards after several months or years of exposure. He considers the specific dynamic action of foodstuffs to be diminished under these circumstances. His observations include data on both natives and Europeans and show no marked contrasts between them, but conclusions are hard to draw since according to other workers strong racial differences in metabolic levels appear to exist (Macleod, Crofts and Benedict, 1925; Earle, 1926).

A secondary chemical regulatory mechanism, consisting of a fall of metabolism below the basal level in response to warmth, has been described by Plaut and Wilbrand (1922). They note that after exposure of men or dogs to high temperatures which produce a rise in rectal temperature, there is a period in recovery during which the metabolism sinks to a subnormal level. They consider this due to a definite chemical regulation against heat which they suppose commonly present, though often masked by other factors. Thus they found an increase of 32 per cent in oxygen consumption as the result of the hyperpnea induced by warming the blood flowing through the carotid arteries in a dog; when, however, the whole animal was warmed no such increase in oxygen consumption was seen, even though the hyperpnea was still in evidence; they suppose that the diminution in oxygen consumption resulting from the secondary chemical regulation balanced the increase necessitated by the more rapid respiratory movements. Deductions

of this type are, however, dangerous, since they have to be made from data obtained in short experimental periods and from animals not in either thermal or chemical equilibrium.

Character of metabolism on exposure to warmth. The tendency of individuals living in a warm climate to avoid high protein diets with their marked specific dynamic action and to choose a diet richer in carbohydrates is well known. Fats are also less palatable in warm climates; they suppy a large heat value in a small bulk and are therefore less adapted for giving a normal stomach distention under conditions where a small number of calories are required; they also do not allow quite such a high standard of muscular efficiency (Krogh and Lindhard, 1920) as do carbohydrates. Nothing is known of the mechanisms by which the appetite adjusts itself so that the more suitable diet is chosen.

Deductions have sometimes been drawn (e.g., McConnell and Yaglo-glou, 1925) from the variations in the respiratory quotient in acute experiments as to the changes in fat and carbohydrate metabolism during the experiment, since respiratory quotients above 1.0 or below 0.7 are commonly observed. All such deductions are absolutely unwarranted, since thermal equilibrium is not established, and complicated adjustments in acid-base equilibrium are certainly being made, so that any complete analysis is at present quite impossible. Some observations have also been made on protein metabolism by Graham and Poulton (1912), who found that a rise of rectal temperature to 40.2° induced by exposure to warm moist air did not necessarily produce any increased breakdown of body proteins; if a diet of high calorific value were taken, the increased energy production set up by this high temperature was taken care of without change in protein metabolism, but if the diet also contained a large protein excess then protein might also take a small share in the increased energy production.

Mechanism of metabolism changes. The increased metabolism accompanying raised body temperature may therefore be ascribed simply to the temperature effect on the velocity of chemical reactions, but that accompanying shivering in the response to exposure to cold is generated through reflexes initiated by centers in the brain and executed through the paths of the spinal nerves; it is absent below the level of a spinal transverse section (Sherrington, 1924) and is not seen if the motor nerves have been cut. Chemical regulatory reactions without shivering are, however, thought to occur and to be transmitted through the sympathetic system since they are found if the spinal section is in the thoracic but not if it is in the cervical region (Freund and Grafe, 1912). Freund

and Janssen (1923) find that the oxygen consumption of the lower limb muscles is not raised by a rise in the muscle temperature of a moderate degree if the thoracic cord (about the seventh segment) has been cut, but that no such independence of the local temperature is observed if the cervical cord is cut. They find that the control of chemical activity thus indicated is not affected by section of the sciatic, but is affected by removal of the periarterial nerve plexus, and they suppose a local reflex with local sensory and motor elements. The results are difficult to explain on other grounds, since as a rule no great alteration in blood flow was observed, and, though considerable acid-base adjustments must have been necessary under such circumstances, it is not clear how they would complicate the results. Such hypotheses and their support of the "second chemical regulation" in response to warmth must however be regarded as *sub-judice* for the present. In addition to these various mechanisms, variations in activity of the suprarenals and thyroid seem probable, as has been already pointed out.

Respiration. The effects of warmth on the respiratory movements of man and the smaller animals are somewhat different, since the latter commonly use these movements as a cooling mechanism instead of sweating. The reaction in animals will therefore be considered separately.

In animals, such as the dog and the cat, the respiratory movements become very rapid and very shallow in response to warmth, thus allowing the air in the dead space to be moved frequently back and forth with consequent maximal evaporation of water without the alveolar ventilation being necessarily increased. Richet (1898) has investigated and described this mechanism; he ascribes the initial response, on exposure for instance of a dog to the rays of the sun, to a cutaneous reflex, since it commences at once while the deep body temperature may be temporarily falling; he considers the 5th cranial nerve particularly important; if the reflexes are depressed with chloral this reaction may be diminished, but, when a rise of rectal temperature is induced, there ultimately occurs at a rectal temperature of 41.7° a very rapid respiratory rhythm, a polypnea which he considers to be of central origin. While it seems probable that both reflex and central factors are concerned in the polypnea their absolute separation into these two types is perhaps artificial, yet unquestionably Richet's figures do show a very sudden change at a rectal temperature of about 41.7°C. Richet described respiratory movements as rapid as 416 in dogs with a rectal temperature of about 42°. That the temperature of the central nervous centers is of great

importance seems indicated by the recent work of Heymans and Heymans (1926) on the isolated head. They find that such a preparation, after removal of most sensory impulses (except those of the vagi and cranial nerves) by section of the neck, shows an apnea, but, if the blood stream to the isolated head be warmed, respiratory movements reappear, become regular at a temperature of about 38° or 38.5°, and may reach a rate of 130 at temperature of 43° or 44°. If sensory impulses were not concerned as a factor in normal polypnea, a respiratory rate higher than this might be anticipated. Polypnea may, however, be seen even after division of the vagi (Richet).

High respiratory rates may also occur in cats; Uyeno (1923) has described respiratory rates as high as 400 in urethaned cats heated in a bath until the rectal temperature reached 42°.

The polypnea seems to be partly dependent on cerebral centers which lie cephalic to the medulla oblongata, judging by the effect of decerebration. Sherrington (1924) finds that in acute decerebrate cats and dogs the respiratory rate is increased when the body temperature is raised, but not to the extent observable in the intact animal, even though a rate as high as 75 was noted in one case with a rectal temperature of 41.1°. Bazett and Penfield (1922) reached similar conclusions in experiments on chronic preparations of the same type; they observed a maximum rate of 60 at a temperature of 42.8°.

It seems probable that many different factors are normally concerned, among them the effect of temperature on chemical processes. Britton (1922) has determined the effect of cooling on the respiratory rate of cats anesthetised with chloroform and ether; the diminution in respiratory rate was progressive, the rate being reduced to 17 at a rectal temperature of 26.9 and the reduction in rate showed a fairly uniform relationship to the temperature giving on calculation a Q_{10} value of about 1.6. But unfortunately the condition is even here not at all simple; when the same animals were allowed to cool further without anesthesia, and then to recover from the cooling, quite different respiratory rates were obtained at the same rectal temperature according to the direction of temperature change. Thus in one animal at a rectal temperature of 20.7° the respiratory rate was 11 when the temperature was falling, and 20 when it was rising. Calculations gave a Q_{10} value of about 2.5 when the anesthetic was discontinued while the temperature was still falling, but during warming it was much higher, very variable, and the results do not seem explicable on any such simple rule. Thus comparison of the initial and final respiratory rates with a rising temperature (19.0°

and 39.4°) in one animal gives a Q_{10} of about 1.7, similar to that seen on cooling, but during the warming process the respiratory rate quickened rapidly at first, and over certain temperature ranges values as high as 7 or 8 might be calculated for Q_{10}. Similarly the values obtained by Bazett and Penfield on decerebrate cats showed considerable fluctuations and, though a minimum value for Q_{10} of about 4.0 or 5.0 was calculated, it seems more probable that a high value of 8 or 9 often more nearly represented the relationship under the conditions of their experiments. Temperature coefficients of this high order were previously neglected and considered due to extraneous factors, but similar coefficients may undoubtedly occur in some other changes, such as those in pulse rate. The occurrence of such high values varying with the direction of the temperature change demonstrates the presence of complicating factors of considerable magnitude. Further data are needed with careful control of the direction of temperature change, and it should be recognized that experiments in which the carotid blood stream is warmed (such as those of Kahn, 1904, or Heymans and Heymans, 1926) cannot be regarded as conclusive evidence of central effects, unless precautions are taken to exclude the influence of sensory impulses through the cranial nerves.

The increased respiratory movements, even though shallow, may produce changes in the blood; thus in the experiments of Flinn and Scott (1923) exposure of dogs to a temperature of 40° only increased the rectal temperature 1° within a 6 hour period, yet the blood alkali was diminished, with a corresponding fall in CO_2 tension, so that the plasma pH was not altered. When, however, they exposed the animals to environmental temperatures of 45° to 50°, there was a marked fall of both blood alkali and CO_2 tension; the animal appeared dyspneic and in this case the blood plasma became very alkaline (increase of 0.22 to 0.25 pH). Recalculation of the data using revised constants (Austin and Cullen, 1925) would only slightly modify the results.

In man there is no evidence of any heat polypnea of the shallow type adapted for cooling. The respiratory rate may be increased with hyperthermia; Sutton (1909) in hot room experiments noted an increase in respiratory rate of some 5 to 6 respirations per minute per degree increase in rectal temperature; with fever patients a similar increase in rate is commonly noted. Such an increase if calculated on a Q_{10} basis would give a value for Q_{10} of 7 or 8; any such change in rate is more comparable therefore with the smaller change of the decerebrate cat than with the polypnea of the intact cat or dog. When, however,

the rise of temperature is rapid, a very definite hyperpnea is also seen in man, but in this case the respirations are very deep (tidal air up to 2 liters), the rate may be at first even slightly below normal (Landis and co-workers, 1925), and the movements appear more designed to produce a maximum alveolar ventilation, than to facilitate evaporation and loss of heat. The increased respiration is accompanied by a definite sensation of air hunger.

Observations (Bazett and Haldane, 1921; Bazett, 1924) have shown that the respirations may become quieter, if the rise of body temperature is checked, even though the actual temperature remains at a high level, and have demonstrated the importance of the rate of rise of temperature. The experiments of the Landis group (1925) have on the other hand shown that a rise of temperature at any given rate is much more effective the higher the absolute temperature of the subject; both rate of change and the temperature level appear therefore to be factors. The hyperpnea causes a marked fall in alveolar CO_2 tension which was first demonstrated by Haldane (1905). Hill and Flack (1909) reported CO_2 values as low as 3.07 per cent, Bazett and Haldane (1921) tensions as low as 25 mm. as the result of an 18 minute exposure to a hot bath, and Landis and his co-workers (1925) calculated tensions as low as 16 to 23 mm. from analyses of respiratory samples collected in a spirometer. The response varies in different subjects, and Haggard (1920) only observed a fall of some 5 mm. in alveolar CO_2 tension in himself in hot baths prolonged for 20 minutes.

Haggard (1920) estimated the alkali present in the blood in his experiments and found only minimal changes, so that he concluded that the pH of the blood must be increased. Koehler (1923) estimated both factors and demonstrated that both in febrile cases and in experimental hyperthermia the diminution in alveolar CO_2 tension is accompanied by an alkalosis, and similar deductions were made by Cajori, Crouter and Pemberton (1923). Further data are given by Landis and his co-workers, who have reported in hot bath experiments rises in pH of the blood plasma varying between 0.12 and 0.33, when the determinations were made by Cullen's method.

Undoubtedly the respiration increases enormously in depth and results in an increased total ventilation at a time when the blood is markedly alkaline, and this change in respiration cannot be readily accounted for on Gesell's hypothesis of the acid metabolism of the center (1925), since the increase is not entirely dependent on the temperature level, and seems too extreme to be attributed to the effect of temperature

on the rate of chemical reactions. It does not seem easy to attribute it to a temporary failure of the circulation to the medulla (cf. some dyspneas described by Gesell), since the blood pressure may be well maintained and the subject be in the recumbent position, and yet the hyperpnea may be very marked (Bazett, 1924). Large volumes of CO_2 are given off as is indicated by the increased respiratory quotients, often much above 1.0, observed by all workers. It is true that the respiratory changes are somewhat irregular and periods with subnormal quotients may also be observed (Koehler, 1923), but the other condition is the more common, and appears to be the primary response. The data of Austin and Cullen (1925) make it essential that increased CO_2 should be eliminated, as the temperature rises, to prevent the blood from becoming more acid, but such an explanation would not account for the blood actually becoming more alkaline, both in men and dogs. When dogs are cooled, until shivering no longer occurs, very low respiratory quotients may be observed and be maintained for hours, so that simple acid-base adjustments do not readily afford a full explanation of the changes in respiratory quotients (Finney, Dworkin and Cassidy, 1927).

Koehler (1923) has shown a maintained alkalosis in febrile patients, indicating a lower threshold for the respiratory center at the higher temperatures. The results of heating could then be explained as due to a lowering of the threshold with an increased metabolism of the respiratory center, especially if a readjustment of buffer substances also causes an actual liberation of CO_2 in the center while it is being warmed.

Even in frogs there is some indication of a similar phenomenon for Vernon's figures (1894) show that the CO_2 output in these animals varies with the temperature, but that the output of CO_2 is greater for the same temperature when the animal's temperature is rising than when it is falling, while the output is greatest of all when the temperature is rising rapidly.

The recent work of Austin, Sunderman and Camack (1927) demonstrates that when a cold-blooded animal is warmed considerable amounts of lactic acid may be formed, so that the condition appears different from that in mammals; the blood of the whole animal in the species investigated (alligator) became more alkaline, when the animal was kept cold, and more acid when it was kept warm, while the amount of base bound by protein remained nearly constant.

Circulation. *Pulse rate.* Any change in temperature affects the pulse rate of the isolated perfused heart and also alters its rate in the intact animal. Values obtained on the excised heart have already been

alluded to and some references given (p. 546); in addition to these Feldmann and Clark (1924) have calculated results obtained on the excised rabbit hearts according to Arrhenius' equation, and they find that a fairly constant value for μ of 14,800 may be obtained. Britton (1922) has given values for the pulse rate in cooled cats; if these be utilized for similar calculations much bigger variations are observed, as might be expected considering the other factors introduced, but the mean value for μ is about 13,500, though it is rather above this value when the temperature is rising, and rather below it when it is falling. Uyeno (1923) gives a few pulse rate figures for cats with rising temperatures in hot baths, and these may be used for similar calculations. The results are very variable but the mean value for μ is about 16,500. Figures obtained by Heymans (1919) give variable values for μ with a mean of about 11,200. Kuno (1914) and Kirsch (1923) have investigated the changes in pulse rate with temperature and have compared the effects observed before and after section of the vagal and sympathetic fibres to the heart. They find no constant difference between the innervated and denervated heart, and consider the only important factor to be the change in temperature of the heart. When one considers the reflex control of pulse rate in connection with venous and arterial conditions (Anrep and Segall, 1926), and the adrenalin effects on the denervated heart described by Cannon and his co-workers (1927), the changes are more remarkable for their uniformity than their irregularity. There is always a tendency for the pulse rate to be more rapid when the temperature is rising then when it is falling, both in animals and man. Though these differences are not very marked, they may be noted in Britton's figures, as well as in curves given by Adolph (1924) and by Bazett (1924).

In man the pulse rate changes are much greater and the conditions seem more complicated than in animals. The figures given by Hill and Flack (1909) for subjects lying down in hot baths show a mean increase in pulse rate of 44 beats per minute for a rise in rectal temperature of 2.4°. On standing the pulse rates became much more rapid. Bazett (1924) with similar baths found a mean increase of 37 beats per minute for a rise of 2°, and Vernon (1923) described increases of the same order of magnitude with the subject standing in a warm room, though the actual rates were somewhat faster. McConnell and Houghton (1923) give many values for subjects sitting in hot rooms, but found no direct correlation with body temperature. From graphs given by McConnell and Yagloglou (1925) of data from similar experiments a relationship

may be calculated giving an increase in rate somewhat greater than the other values already quoted. Adolph (1924) notes a correlation with rectal temperature in warm room experiments only after establishment of thermal equilibrium. He considers the correlation better with mouth temperature than with either surface or rectal temperatures, but it is possible that his results might be better explained by correlation with two separate conditions, one blood temperature and the other rate of change of temperature; in acute hot room experiments temperature conditions are further from equilibrium than at the end of hot bath experiments. Calculations of temperature coefficients from these data give values of 7 to 12 for Q_{10}, and values for μ in Arrhenius' equation of 40,000 or more. The values appear to be real, however, for the smaller increases in pulse rate described correspond closely with those commonly observed in febrile conditions. The factors concerned in man and animals are therefore not identical. A possible explanation could be a greater importance of reflex effects on the heart rate in man than in animals, which do not have to contend normally with an upright position. In man there is rarely any exact parallelism between rectal temperature and pulse rate, so that the effect of temperature changes on the heart muscle itself does not provide a complete explanation of the data.

Circulation rate. Bornstein (1911) investigated the character of the pulse curve by the tachogram method, and concluded that the minute volume output of the heart was increased by cold and hot baths, with an increased stroke volume and slowed pulse in the former case, and the reverse combination in the latter. Schapals (1912) made estimations of the flow by the use of the Fick principle and came to similar conclusions, though he considered that a decrease in stroke volume almost balanced the increased pulse rate with warm baths under 40°, so that the minute volume was then little changed. Lindhard (1915) reported observations on the effect of cold and warm baths on the circulation; his observations commenced 3 minutes after the subject entered the bath, which was commonly below 20° or above 40°. He found an increase in pulse rate in the warm baths, often a slight decrease in stroke volume, but a definite increase in the minute volume; in the cold baths the pulse rate was slower than normal, but through an increase in the stroke volume the minute volume was sometimes increased.

Barcroft and Marshall (1923) employed the Fick principle and experimented in cold and warm rooms; in both the minute volume was increased, and they obtained further evidence of the increased stroke volume with exposure to cold. Uyeno (1923) applied the Fick principle

to cats, employing heart puncture, and found a considerable increase in the minute volume in hot baths. All these workers found the increase in minute volume due to warmth to be caused mainly by the change in pulse rate; their figures often show no significant change in stroke volume, or variations in the direction of the change in successive experiments. It seems probable that the direction of change on exposure to warmth is not constant. Heymans (1919) experimented on unanesthetized rabbits with a carotid-jugular anastomosis and under these conditions observed with warmth an increased output from the carotid cannula, which was greater than could be accounted for by the change in pulse rate, and which appeared to indicate an increased stroke volume.

Local vasomotor changes. When any part of a skin surface is exposed to temperature changes, there occurs a vasodilatation in response to warmth and of vasoconstriction to cold. Inspection demonstrates very considerable changes in the veins, the skin becomes red when exposed to heat, and capillary pulsation may be observed (Lewis, 1924). A rise of temperature causes local dilatation of the capillaries (Krogh, 1919), but the capillary pulsation is probably mainly the result of arteriolar dilatation, which is also demonstrated by an increased volume of the part of the body warmed (Amitin, 1897), and by a more rapid local circulation rate (Stewart, 1911; Hewlett, van Zwaluwenburg and Marshall, 1911; Carrier, 1922).

To some extent the above effects appear to be produced by physical or chemical means. Arterial strips have been demonstrated to relax when warmed in normal saline; Cow (1911) repeated previous work and found that such a strip showed decreased tonus when warmed from 12° to 45° or higher, but that in some vessels, e.g., the carotid, some slight recovery of tone might be noted even with a rising temperature at temperature ranges a little below those of deep body temperature. A somewhat different description is given by Cruickshank and Rau (1926). Lewaschew (1881) found that the flow of defibrinated blood perfused through limbs, in which the nerves had been previously allowed to degenerate, was greater the warmer the perfusing blood. Goltz and Ewald (1896) removed completely the lower segments of the cord in dogs and found that the resulting vascular dilatation disappeared in a few days, that after this the foot pads might even be colder in the limbs affected than in the normal front paws, and that the affected parts showed normal vascular responses to temperature changes. If one sciatic nerve was later divided in such an animal, the foot on that side at first became much redder and warmer than the other, but later recovered its

tone and was even colder than the hind foot affected by the initial operation. In cats the conditions seem somewhat different, for Dale and Richards (1918) observed that, after complete degeneration of the nerves, the pads of the feet were paler than on the normal side, but were warmer and gave up heat more rapidly to cold water. They attributed this result to arteriolar dilatation with capillary constriction. As, however, the arterioles of the cat constrict with histamine, while those of the dog and monkey dilate (Burn and Dale, 1926), the arterioles in the cat are somewhat peculiar and the differences observed may depend on the species used.

Trotter and Davies (1909) after nerve section in man noted a marked redness and increased surface temperature in the affected area during the first few days, but found that, after this time had elapsed, the condition changed to one where the difference was slight but in which the surface temperature of the affected area was slightly higher in a warm, and lower in a cold room, than that of the surrounding normal skin. Evidence that a chemical stimulus may be concerned even in the production of reflex arteriolar changes has been brought forward by Lewis and Grant (1924) in their investigation of reactions to injury, and they advanced evidence that such a chemical stimulus was concerned in responses to temperatures above 43°. These ideas have been further developed (Lewis, 1926; Lewis and Love, 1926; Lewis and Marvin, 1927) and the hypothesis has been advanced that the reddening of the skin, which may accompany surface temperatures even as low as 36°, may also be initiated by some substance of the histamine type, since, if the circulation be temporarily arrested and later released, a delayed heat hyperemia may be seen even in a limb which has in the mean time been cooled. Further a heat hyperemia may fade more quickly in a limb kept moderately warm than in one kept cooler. Even the action of vasodilator nerves has been considered as effected by chemical means (Lewis and Marvin, 1927). Goldblatt (1926) showed that the somewhat similar reactive hyperemia, which follows a temporary arrest of the circulation, can be demonstrated after the section of the nerves to the part. It was pointed out by Lefèvre (1911) that the skin becomes red and hyperemic when it is exposed both to cold and warmth. These conditions have been investigated by Goldschmidt and Light (1925b); they find the dilatation of the superficial vessels with cold to be accompanied by evidence of a very slow blood flow, and they consider that the dilatation might conceivably be due to a paralysis of the capillary mechanisms, and the bright red colour to diminished metabolism or

dissociation of oxyhemoglobin and consequent lowered use of oxygen. With heat they find (1925a, b) a considerable dilatation with evidence of a very rapid blood flow, particularly with immersion of a part in water above 45°. In some of their experiments with low temperatures a swelling of the cooled part was noted, and a swelling was also constantly observed after exposure to the higher temperatures.

Thus there is a considerable amount of evidence in favour of such reactions to temperature being produced in part by mechanisms other than those of the nervous system, and it seems probable that with exposure of the skin to water over 43°, possibly even over 41°, inflammatory reactions of the histamine type may be a factor; at temperatures below this such a mechanism is less clearly established. The facts that have been already stated in regard to acid-base equilibrium (p. 547) make it evident that the mere warming of a peripheral part can increase the acidity of the blood supplying it (if the acidity in the central vessels remains constant), and might therefore act as a chemical stimulus to vasodilatation, for the considerable effect of pH changes on vascular tonicity has often been demonstrated (Leake, Hall and Koehler, 1923, who also give references to work of Gaskell, Bayliss, Hooker, Fleisch, etc.). But if this be the main cause, proteins with buffer properties similar to those of the blood proteins would presumably have to exist in the vessel walls to explain the results on excised vessels. The decreased acidity demonstrable by dye methods in cooled limbs might also be due to depression of oxidative changes by the lowered temperature, but, however caused, they occur in spite of the demonstrable vasoconstriction and decreased circulation rate, which may even prove to be to some extent protective mechanisms designed to maintain an acidity as near normal as possible.

The local alterations in vasomotor tone produced by temperature changes are probably obtainable in most, if not in all, vessels, and are not confined to the skin, for Hess (1912) demonstrated constriction of the pulmonary vessels to cold and Florey (1925) and Schmidt (1926) have shown that the cerebral vessels react both to warmth and to alterations in acidity, even though the ordinary vasomotor reactions are absent.

But though direct or semidirect actions appear to be important, and particularly in denervated areas, they do not seem to be the main factor in the normal response. Luchsinger (1877b) obtained evidence of an active vasodilatation in the response to warmth, for the vessels in a normal foot pad might be more dilated under such circumstances than in another, the nerves to which had been recently sectioned.

Sherrington (1924) has shown that after complete spinal transection in dogs the skin of the affected area remains warmer than that of control animals, and that, though some vasomotor tone is regained and pressor spinal reflexes may be elicited, yet the vascular responses to temperature show a persistent failure, which "though not absolute remains severe." For instance the surface temperature on the pinna of a normal dog fell in a cold room from 34.0° to 20.2°, while that of a dog with a cervical spinal lesion only decreased from 35.2, to 32.2°, even though the vaginal temperature had fallen 2.2°. The contrast between normal and abnormal reactions is therefore the exact opposite of that seen in denervated skin.

An indicaton of the importance of nervous reflexes is also supplied by the somewhat paradoxical temporary vasoconstriction that may occur in response to extreme warmth. This has been noted even in the part exposed to the temperature change, if this is sudden (Amitin, 1897; Uhlenbruck, 1924). Such sudden increases in temperature may also stimulate cold spots, but it is not known whether any such stimulation is a necessary precursor of the vasoconstriction.

It would appear, therefore, that under normal conditions the vascular changes are induced by nervous reflexes evoked through centres in the brain rather than in the spinal cord, and that, in the absence of such reflexes, no pronounced temperature change is readily induced, so that neither physical nor chemical direct effects are important; once, however, the reflex changes are induced, the temperature changes are profound and the circulatory condition may possibly be maintained or even exaggerated by direct physical or chemical action. In the denervated skin the conditions seem modified; it has been shown by Dale and Richards (1918) that, after nerve degeneration has set in, the vessels become hypersensitive to adrenalin and histamine, and the exaggerated response to temperature (described by Trotter and Davies, 1909, and detectable in the experiments of Goltz and Ewald, 1896) could therefore be attributed to such a hypersensitiveness, either to acid-base changes, or possibly to histamine itself.

Temperature changes produce not only local but also *general vasomotor responses*, which in many ways resemble the local reactions. Thus Loewy and Dorno (1925) have demonstrated that, if the hand be placed into cold water, a fall of surface temperature due to vasoconstriction may be demonstrated on the chest. Stewart (1911) has shown that the rate of circulation through the hand or foot may be decreased by placing the opposite limb in cold water, or be increased if warm water is

used, and these results have been confirmed by Hewlett and his co-workers (1911). Briscoe (1919) demonstrated that a transient fall of pressure may occur in both veins and capillaries in one hand, when the other was plunged into cold water, and a visible contraction of the veins might also be seen. Goldschmidt and Light (1925b), using plethysmographic methods, found that such reflex responses in one arm to temperature changes in the other were much affected by the temperature of the water surrounding the arm in the plethysmograph; if the water was unusually warm or cold, crossed effects could not be demonstrated from the opposite arm. Their provisional explanation of such results was that the condition of the blood vessels in a distant area is determined by the algebraic summation of reflexes resulting from a number of sensory impressions. In their experiments with the arm immersed in water at 29 to 34°, the blood flow seemed to be much influenced by the room temperature, which, affecting nearly the whole body surface became an important factor. Such reflex vasomotor responses in distant areas are not confined to the skin; very active reflexes of this type have also been demonstrated in the mucous membranes of the pharynx, etc., (Mudd and his co-workers, 1919, 1921) where the temperature changes resulting from vasoconstriction may long outlast those demonstrable in the skin. Kahn (1904) gives evidence also of central vasoconstriction to warmth.

It is uncertain from which end organs these reflex responses in other regions originate. One might anticipate that the cold and warm spots are concerned, but a case has been reported by Stewart and Walker (1913), in which reflex changes in the blood flow of the right hand occurred, when the fingers of the other hand were inserted in hot or cold water, although these fingers were anesthetic to both warmth and cold as the result of a previous peripheral nerve lesion. The latent period of the response was of about normal duration; conceivably more deeply situated end organs are concerned in such responses. The initial vasoconstriction which may occur locally in response to very warm stimuli may also be demonstrated in the response at a distance (Amitin, 1897) and similar reactions have been noticed by other workers (e.g., Stewart and Walker, 1913).

Blood pressure changes occur as the result of the interaction of the alterations in circulation rate and the vasomotor changes.

Exposure to cold with both vasoconstriction in the skin and an increase in the minute volume of the heart output is usually associated with a rise in blood pressure, even though the vessels supplying the

muscles are probably dilated if the muscle metabolism has been increased. Edgecombe and Bain (1899) noted this rise in arterial blood pressure as the result of cold baths and found it to be accompanied by a fall in venous pressure. Strasburger (1905) found the arterial systolic pressure rose initially in a cold bath, fell again a little later and afterward rose a second time.

With exposure to warm air or baths the changes in arterial pressure are less constant, since an increased heart output is accompanied by a skin vasodilatation. Warm baths (35 to 40°) cause a considerable fall of systolic blood pressure (Edgecombe and Bain), while the diastolic pressure shows a similar but less noticeable fall (Strasburger). In experiments on anesthetised rabbits Kuno (1914) found that warm baths initially lowered the mean blood pressure, but that within a few minutes the pulse rate increased and the pressure rose above normal value. Cold baths also increased the mean pressure while slowing the heart rate. He ascribed the effects partly to the skin stimulus (most of the warm baths had temperatures of 40° or more) and partly to the hydrostatic pressure of the water. Hill and Flack (1909) reported very considerable falls in the systolic pressure level as the result of warm baths, and Bazett (1924) noted a moderate fall in both systolic and diastolic pressures, especially diastolic, under such circumstances.

With exposure to high air temperatures Sayers and Harrington (1921, 1923) have described lowered systolic and diastolic pressures, and the systolic pressure was the more affected in some cases, the diastolic in others. Other workers (McConnell and Houghton, 1923; McConnell, Houghton and Phillips, 1923; McConnel and Yagloglou, 1925) have noted a marked fall in diastolic pressure with a rise in systolic pressure on exposure to warm air. The diastolic pressures reported by this group of workers cannot, however, be taken as indicating more than the direction of change, since they constantly reported values of "minus zero" in warm conditions, and do not appear to have observed the necessary technical precautions. Adolph (1924) also notes a rise of systolic pressure in warm rooms with very low diastolic pressures, and some zero values are recorded. Such low diastolic values seem unlikely to represent the real conditions within the vessels. He noted, as did Hill and Flack (1909), the difficulty with which an erect posture is maintained when the peripheral vessels are so profoundly dilated, and observed a considerable relief of symptoms if the legs were raised.

The rise in systolic blood pressure noted by so many observers in hot air experiments may be also seen in hot as opposed to warm baths; if the

temperature of the water is over 40° Strasburger (1905) noted a systolic pressure above the initial level throughout the duration of the bath, and Bazett (1924) also described occasional rises of systolic pressure even in warm baths (less than 40°) if the bath was prolonged. Heymans (1919) described a rise in systolic and a fall in diastolic pressures in rabbits, when their temperatures were raised by warming the blood flowing in a carotid-jugular anastomosis.

The varying blood pressure response to warm and hot air or water temperatures would, therefore, appear to be dependent on the degree of vascular constriction or dilatation, which may in turn be modified by the amount of skin stimulation, on the degree of alteration of heart output, and its proportional relationship to the vasomotor changes, and on the change in pulse rate, since with any given minute volume a high pulse rate is accompanied by a smaller stroke volume and pulse pressure, so that both diastolic and systolic pressures more closely approximate the mean pressure level. The position of the subject influences venous return, and so heart output and arterial pressure, and also causes variations in the differential distributions of the blood to the different parts of the body.

Edgecombe and Bain (1899) found that venous pressure was reduced by both hot and cold baths, as well as by Turkish baths.

BLOOD VOLUME, FLUID INTERCHANGE AND WATER EVAPORATION. On exposure to continued warm conditions an increase in *blood volume* may be demonstrated, though it appears to be induced relatively slowly and the maximum effect may not be noted until one or two days have elapsed (Barcroft et al., 1922). Their data were obtained by the carbon monoxide method, both in the tropics and in an artificially warmed room in England, and demonstrated increases in blood volume of at least 10 per cent and probably 25 per cent or more. In the room experiments initially a dilution of the blood occurred without an increase in the total oxygen capacity, but later, and noticeably after exercise, the increased blood volume was associated with a marked increase (at least 10 per cent) in the total oxygen capacity of the circulating blood. In one subject after two days under artificial warm conditions an increase in reticulated red corpuscles was noted, in another subject this was absent but the subject was unable to withstand the higher temperature and became ill.

In experiments of shorter duration, blood volume changes of similar type have been inferred from alterations in the concentration of red cells, hemoglobin, total solids, or changes in the viscosity or specific

gravity of the blood, all of which have given indications of the passage of fluid into the blood as the result of exposure to warmth. Since, however, the circulating red cells cannot any longer be legitimately assumed a constant quantity, such alterations, while useful as an indication of the direction of change, cannot be regarded as an adequate method of measurement of fluid interchange.

Kestner and Gross (1919) demonstrated in both men and animals a decreased hemogobin concentration, increased plasma protein and a normal salt concentration in the blood in the early stages of adaptation to the warmth of exercise (in climbing) which was accompanied by sweating. In later stages, particularly if the salt supply was inadequate, a blood concentration might be observed. Cohn (1919) confirmed these results under laboratory conditions. Eckhert (1920) obtained similar reactions to warmth, both with and without exercise; he made experiments with hot air and hot baths, and noted that with extreme conditions (baths 42° to 45°) an increase in blood concentration might occur quite early, while with less acute conditions the previously described decrease was at first observed. Barbour (1921) has emphasized these changes. He and his co-workers (Barbour and Tolstoi, 1924; Barbour and Hamilton, 1925) have shown that the alterations in blood concentration are rapidly induced, reversible, and of similar degree in arterial, venous, and capillary samples. The changes were no longer in evidence in dogs after spinal transection at a high level, nor after decerebration. The dilution on exposure to warmth affected not only the whole blood but also the plasma and indicated an increased water content. Borchardt (1926) has recently investigated the effects of sweating on the blood composition in man, and his results agree with those of previous workers, but in addition, he found that prolonged sweating may not only cause a water deficit but also a lowering of the potassium concentration of the blood. This he attributed to the fact that the potassium sodium ratio is higher in sweat than in blood, and he considered the after-effects of prolonged sweating (fatigue and muscular weakness) possibly due to this change in potassium concentration in the blood. Flinn and Scott (1923) confirmed the occurrence of blood concentration in dogs on exposure to extreme heat, but failed to obtain a dilution under milder conditions. Flinn (1924) found that this blood concentration could be prevented if there was a considerable water intake by mouth. Lozinsky (1924) noted that a blood concentration caused by excessive heat may have almost disappeared an hour after the return to a normal environment, even if no fluid is taken by mouth, and he considered this to indicate that simple dehydration is not the only factor concerned.

The sources of the increase in red cells in the blood stream, which Barcroft and his co-workers demonstrated (1922), are rather uncertain except that one of these sources is the spleen. They have shown that the spleen in response to various conditions of strain, including muscular exercise, is capable of a considerable contraction expelling into the general circulation blood containing a high percentage of red blood corpuscles (Barcroft and Barcroft, 1923; DeBoer and Carroll, 1923; Barcroft, 1925). The occurrence of reticulated red cells at the end of one or two days' exposure to warmth in some subjects suggests that the bone marrow may in addition be stimulated to increased production of red cells.

The source of the fluid is also inadequately known. Kestner and Gross (1919) demonstrated in animal experiments that the water content of the muscles had been lowered, and they considered the muscles as the main source of fluid. Probably the intestinal tract contains a considerable reservoir of fluid and salts which can also be drawn upon, though there is no direct evidence of this. The kidney conserves fluid and secretes a concentrated urine. Barbour (1923) has shown that a diminution in brain volume accompanies blood dilution, but such changes may be simply due to vasomotor readjustments. In favour of such an explanation is an initial increase in brain volume sometimes noted when an animal was suddenly immersed in a very hot bath (Barbour, 1924) a change opposite to that observed later in the experiment. This paradoxical result may be related to the initial vasoconstriction response to extreme heat noted by many workers. None the less it is probable that alterations in the water content of the brain are also induced similar to those described by Weed and McKibben (1919). Hamilton and Barbour (1925) have reported experiments suggesting that the fluid entering the blood may in part be derived from subcutaneous tissue.

The cause of the alterations in blood volume would appear to be mainly readjustments necessitated by the vasomotor changes. A vascular dilatation in the skin, even though accompanied by a vasoconstriction in the more central organs (Kahn, 1904) might readily cause an increase in the total capacity of the circulatory system, which would demand a parallel increase in blood volume; the peripheral vasodilatation in the skin in spite of a fall of arterial blood pressure might result in a local rise in capillary pressure, but in the more central organs a combination of a fall of arterial pressure with some vasoconstriction should lower capillary pressure and attraction of fluid by unbalanced osmotic pressure might be anticipated. The fluid interchange of the blood might, there-

fore, be explained in terms of Starling's hypothesis (1909) of fluid balance in favour of which good experimental evidence is accumulating (Landis, 1927). Such fluid interchanges might not be solely dependent on alterations in capillary pressure, since possible changes in acid base equilibrium with temperature may also result in some modification of protein osmotic pressure in the blood or tissues (Austin, Sunderman and Camack, 1927).

The explanation above suggested implies an absorption of fluid in the central parts of the body with little or none in the subcutaneous tissue, where on the other hand filtration might even be facilitated, and fluid be made more readily available for production of sweat or evaporation as insensible perspiration, but such an explanation appears to be at variance with the experimental results obtained by Barbour and his coworkers. They investigated the blood concentration on exposure to cold which they considered dependent not on an entrance of new corpuscles from the spleen but on a loss of fluid from the blood into the cooled tissues themselves; for it was unaffected by denervation of the spleen or by division of the splanchnic nerves (Barbour and Hamilton, 1925a). A larger content of water was demonstrable in the subcutaneous tissue and muscle of the cooled side, when dogs were kept lying on slabs of ice for 20 to 50 minutes immediately before they were killed (Hamilton and Barbour, 1925). They therefore postulated a water interchange due to arteriolar constriction, capillary dilatation from anoxemia and increased capillary permeability (Barbour and Hamilton, 1925b). But such generalizations from their results seem unwarranted at present, for no control observations were made on their dogs to determine the effect of gravity and it is possible that the differences in water content observed might be explicable on this and not on the temperature basis; also, if an animal had a thin coat of hair, an ice block might prove to be an excessive stimulus and one giving results comparable with slight frost bite, rather than with those ordinary cold. In frost bite the results of Lewis and Love (1926) suggest the liberation of histamine-like substances.

It is common knowledge that the limbs (e.g., the feet or fingers) swell during warm weather and shrink during cold weather, and, though these changes may be to some extent dependent on the blood content, they progress too slowly to be explained satisfactorily entirely on this basis. Direct evidence of an increased permeability of capillaries in an area subjected to local warmth has been given by Okuneff (1924). Barbour's hypothesis cannot therefore be considered as established at the present time.

The value of the water interchange is considered by Hamilton and Barbour (1925) to be the production of a better insulation on exposure to cold. This they think is brought about by a thickening of the superficial layers through the deposition of minute droplets of tissue fluid, though they consider that the diminution in the blood volume is also important, since it facilitates the reduction of the peripheral blood flow and heat retention. But Lefèvre (1911) quotes data from other workers which indicate that an increased fluid content decreases the insulating value of excised skin. The well recognized prolonged diuresis that may be noted in man, when the weather shows a sudden change from warmth to cold (e.g., in the mountains where artificial heat is not available), suggests very strongly the presence of large amounts of fluid useless to the body under the new conditions. It is unlikely that some means of fluid retention would not have been devised if water was required for insulation, so that the adjustments of the blood volume to the optimum volume for the circulatory system at the moment would seem the more reasonable explanation of the fluid interchange. There is indirect evidence (susceptibility to burns, character of skin on cold day, etc.) that a dry cuticle has a better insulating capacity than a moister one, particularly if the skin becomes rough, so that air movement on its surface is hindered; should the conception of Hamilton and Barbour prove true, distinctions might therefore have to be drawn between different layers of the skin.

Further evidence that it is the vascular condition which demands fluid interchange to readjust the blood volume is supplied in the experiments of the Barcroft group; high temperatures seem to demand a permanently large blood volume, which, though at first achieved by blood dilution, is ultimately maintained with a normal blood concentration through the mobilization of erythrocyte reserves. The experiments on spinal transection of Barbour and Tolstoi themselves also agree best with such an hypothesis.

The whole question of fluid interchange is also much affected by *water evaporation*, for 1 liter of water requires approximately 580 kilogram calories for its vaporization, so that this factor is of extreme importance in regulating heat loss; loss of water (and salts) incurred in this way is doubtless partly, if not wholly, responsible for the blood concentration seen after long exposure to heat.

The loss of fluid from the respiratory tract is a factor in heat loss in man, even though it is not used as the main means of control. According to Galeotti (1912) the expired air normally contains about 0.0342 gram of

water per liter, corresponding to a saturation of 78 per cent at 37° or complete saturation at 32.5°. The temperature of expired air varies between 30° and 35° with a water saturation of 98 per cent according to Liljestrand and Sahlstedt (1925). Galeotti found the water content of expired air somewhat greater when the subject was feeling warm; this might be anticipated from the vasomotor changes in the nose and pharynx demonstrated by Mudd and Grant (1919). The heat so lost through evaporation by a man during quiet respiraton of moderately dry air might amount to 4 or 5 large calories per hour and be proportionately greater the more active the respiration.

In animals this may be used as the main controllable factor in heat loss, though even in small and furry animals insensible perspiration is also a definite factor.

Insensible perspiration includes not only evaporation of fluid derived from the sweat glands, when the rate of evaporation is greater than that of secretion so that no visible collection of fluid results, but also evaporation on the body surface of water from the tissue fluids and the blood stream itself. This evaporation may be considerable even in animals without sweat glands. Keeton (1924) found that rabbits could not maintain a normal temperature by evaporation of moisture from the respiratory tract, if loss of heat from the body surface was prevented, even though their respiratory tracts were supplied with plenty of cool dry air. The heat lost from the rest of their surface was demonstrated to be considerable, was affected adversely by humid conditions, and was probably under vasomotor control, particularly on surfaces such as those of the ear, which have long been recognized to be concerned in temperature control in these animals. A considerable evaporation of moisture from the whole skin surface seemed indicated, and would be in agreement with a similar evaporation of moisture demonstrated by Loewy and Wechselmann (1911) in subjects with congenital absence of sweat glands, who were found under normal conditions to lose as much water from the skin as did normal subjects. Moog (1922) considered that the insensible perspiration was under special control, since at a room temperature of 25° he found that the loss was often greater the higher (within limits) the humidity of the air. He thought (1924) the regulation could not depend entirely on vasomotor adjustments, since the local vasodilatation accompanying inflammatory reactions may be associated with a diminished water loss, so that he ascribed the control to activity of the sweat glands. The low water loss in a complex reaction such as inflammation may, however, ultimately prove to have a simple physical cause.

Benedict (1926) has shown that if the room temperature is high and visible sweating occurs, the fluid loss from the skin is very great, and irregular in amount, but that at lower temperatures it is regular, is proportional at rest to the basal metabolism, and is unaffected by considerable environmental changes such as removal of the clothes, or variations in air movement. This insensible perspiration appears, therefore, to maintain a definite large proportion of the total heat loss under such conditions, and to be controlled in the same, or in a similar manner, as other means of heat loss; this control may be supposed to be through vasomotor changes, in spite of the contrary evidence advanced by Moog.

Sweating. The considerable literature concerned with this subject will only receive brief attention here; other references may be found in the review articles already mentioned (see p. 531). Sweat consists of water with a low concentration of sodium and potassium chloride (chloride 0.02 to 0.085 M), and the concentration of chloride tends to rise to the higher of these values if sweating is prolonged (Adolph, 1923b). The acidity becomes less during such a period of prolonged sweating, though whether the greater alkalinity is secondary to the alkaline change in the blood, or is dependent on a gradual washing out of organic acids from the skin is uncertain. Various values have been given for the pH of human sweat; 5.2 to 6.6 (Talbert, 1919, 1922); 6.5 to 7.3, Adolph (1923b); 6.0 to 6.9, Bazett (1924). Talbert and Adolph have demonstrated that it varies in different parts of the body in the same individual. Unfortunately it is very difficult to obtain sweat without loss of CO_2 through exposure to air, and even in Talbert's experiments, when sweat was collected in rubber bags, the air within the bag probably had a lower CO_2 tension than that of the sweat, and all loss cannot have been prevented. Adolph's values are certainly complicated by this factor. The few values reported by Bazett were obtained by immersing the hands in distilled water under paraffin oil; pH values in the neighbourhood of 7.0 can therefore be obtained even under conditions when CO_2 loss is unlikely to be significant, but in general the more acid figures are likely to be the truer, unless they can be attributed to organic acids derived from the skin. The volume of sweat secreted may be very great, and may exceed 1 liter (1.6 per cent of the body weight) per hour (Bazett, 1924).

Loss of water through the kidney varies in regard to the available water supply after the other needs have been met, so that a decreased secretion of urine commonly accompanies excessive sweating. In hot

baths, however, secretion of water may occur rapidly through both channels, but in this case the diuresis appears due to the peculiar conditions of the hydrostatic pressure of the water (Bazett, Thurlow, Crowell and Stewart, 1924; Griffith and Hansell, 1925). Experiments demonstrating the effect of sweating on urine secretion are given by Garrett (1898) and by Adolph (1923a).

Blood composition. Changes in blood composition have already been referred to in relation to the direct and indirect effects of temperature on acid base equilibrium (pp. 547, 571), and in regard to fluid interchange and alterations in blood volume. Temperature changes also cause alterations in the *blood sugar level.* In cold-blooded animals the blood sugar concentration varies in the same direction as the temperature (Austin, Sunderman and Camack, 1927), but in warm-blooded animals the changes are more complicated. Flinn and Scott (1923) made a detailed study of the effect of temperature on the chemical composition of the blood in dogs; they found the blood sugar concentration to fall slightly when the animals were at rest in a warm environment in which they were able to maintain their body temperature constant, but with higher temperatures, accompanied by a sudden rise in body temperature, the blood sugar concentration rose abruptly. At intermediate temperatures, where the rise in body temperature was slow, the blood sugar concentration at first fell, but later rose above normal. A rise in blood sugar concentration may also accompany a fall in body temperature (Heymans, 1919; Morita, 1921). Lèpine (1918) immersed one leg of a curarised dog in a warm bath and the other in a cold bath, and noted a higher blood sugar concentration in the warmed limb.

Blood cells. The changes in red cells have already been referred to in relation to blood volume. The white cell count is increased by cold baths (Thayer, 1893) and also by sudden application of heat (Winternitz, 1906). The rate of locomotion of leucocytes was found by McCutcheon (1923) to be affected by temperature to an extent similar to that of a chemical reaction; the maximum rate was reached at 40°; above this temperature other factors were introduced and the rate rapidly fell. Madsen and Wulff (1916) found the optimum temperature for phagocytosis to be the normal blood temperature of the species concerned, namely, 37° for man, 39° for a guinea pig and 41° for a bird.

Skin. Heat affects the skin by the production of the alterations in the vascular condition, evaporation of water, sweating, etc., that have already been described. In addition variations occur in the loss of carbon dioxide from the skin and in the condition of the pilo-motor apparatus.

The loss of carbon dioxide from the skin was estimated by Schierbeck (1893) to be in man about 9 grams a day under normal conditions; when the air temperature was raised it was increased to 20.9 grams and 29.5 grams for air temperatures of 33.8° and 38.4° respectively. At air temperatures below 33° it was almost constant; between 33° and 34° there was a very sudden increase, while at still higher temperatures a more gradual increase was seen. A similar increase with rise of temperature was described by Barratt (1897).

The increase in carbon dioxide lost can be partly accounted for by the rise in CO_2 tension which is likely to accompany a local rise of temperature, unless loss of CO_2 can occur freely (Austin and Cullen, 1925), but the sudden change at an air temperature of 33° probably depends on vascular dilatation. Since the metabolism of the skin is undoubtedly low, the tension of CO_2 in these tissues, and consequent loss, must be mainly dependent on the tension existing in the capillary blood, and on the adjustment of the blood flow so as to maintain this tension in spite of loss through the skin. Possibly variations in the permeability of the skin also occur.

Pilo-motor changes occur in the form of "goose-flesh" with erection of the hairs and roughening of the skin in response to cold, but a similar, though fugitive response, may often be noted in the response to extreme heat, as on entering a hot bath (Strasser, 1923). Cooled blood perfused through an excised limb can also produce it, so that in part a local mechanism may be concerned (Bier, 1897).

Testis. The degeneration of testicles which have been retained within the peritoneal cavity in animals, which normally carry them within the scrotum, has been ascribed by Moore and Oslund (1924) to the higher temperature of the peritoneal cavity. They found that wrapping of the ram's scrotum, if adequate to cause a local rise of temperature, resulted in degeneration of the testicles and loss of spermatozoa within a few months. Moore and Quick (1924) demonstrated that the temperature within the scrotum was actually several degrees below that of the peritoneal cavity in a number of different species. Whatever the original cause of scrotal development, it would appear that those species which normally carry the testicles outside the main body cavity, need to develop and store the spermatozoa in cold storage, several degrees below the peritoneal temperature; to aid control of this temperature the dartos and the cremasteric muscles seem to have developed the capacity of considerable variations in tone, so that they are relaxed under warm conditions, contracted under cool, and the cremaster also

contracts reflexly to stimuli, particularly those of cold, applied to the skin of the thigh in the neighborhood.

Muscle. It has long been recognized that considerable changes in temperature must occur in muscles during contraction, even in mammals and the effect of these changes on the local supply of oxygen has been discussed by Barcroft (1914). The figures that have already been quoted also indicate that the temperature of the limb muscles varies considerably with environmental conditions, and the data obtained by Berner and his co-workers (1926) suggest that these variations, as well as the rise in temperature which accompanies exercise, affect such phenomena as those of "second wind." Fulton (1926) has reviewed and critically discussed the literature which deals with the effect of temperature on muscular contraction and it will not therefore be considered here.

Central nervous system. Up to the present time no adequate analysis of the effects of temperature changes on the central nervous system has been achieved.

Heymans (1919) demonstrated that the brain was the part of an animal most sensitive to high temperature; the fatal temperature was in rabbits between 42 and 43°. Heymans and Heymans (1926), in experiments on the isolated head of the dog, found that reflexes might still be elicited when the sublingual temperature of the isolated head was raised to between 45° and 46° but that above this temperature the brain died.

The magnitude of a reflex response may be considerably modified by temperature. Working with the muscular contractions induced reflexly by single induction shocks, Storm van Leeuwen and van de Made (1916) found that the maximum muscular response occurred when the temperature of a spinal cat was about 38°; at temperatures above this the response diminished rapidly, at lower temperatures it was almost constant until a temperature of 35° was reached, but below this it diminished regularly.

The frequency of the spinal discharge is diminished by a fall of temperature. The frequency of discharge of the cell activating the electrical organ of Malapterurus is reduced about $\frac{1}{3}$ by a fall of 10° (Garten, 1910). The frequency of discharge of reflexly induced impulses from the spinal cord of the frog is reduced by a fall of temperature, and the same is true of the spinal cat (Cooper and Adrian, 1923, 1924) even though the exact amount of reduction cannot readily be estimated, since the different nerve fibres are out of phase. The tendency of the spinal cord to give a repetitive discharge to a single stimulus is also decreased

by a fall of temperature (Cooper and Adrian, 1924). The rhythm of the impulses recordable in the human electromyogram may be diminished by local cooling of the muscle (Forbes and Rappleye, 1917), so that alterations in the effector organ may also be of importance.

The threshold value of electrical stimuli also varies with temperature; Lutz (1918) found that the threshold for reflex responses varied with temperature changes about nine times as much as did that for direct stimulation of a motor nerve. Storm van Leeuwen and van de Made (1916) also investigated the threshold value of single induction shocks at different temperatures in the frog; they found two optimal temperatures, even for the same reflex (homolateral flexion), one between 5° and 6° and the other between 15° and 20°. L. and M. Lapicque (1912) investigated the voltage necessary to obtain a contralateral flexion reflex in the frog at any given frequency of stimulation when the temperature of the point of stimulation or of the spinal cord was varied; they found that if the spinal cord was cooled slow frequencies were effective at much lower voltages. Such a result might indicate an easier production of summation in the spinal cord under these conditions and might parallel the effect of temperature on the production of summation in a muscle. This would be in entire accord with the theory of reflex action advanced by Sherrington (1924).

In some of the above experiments, the methods employed have failed to distinguish between the effects of temperature on the sensory end organs, peripheral effector organs and the synapses of the central nervous system, nor is it certain how far responses to single and multiple stimuli differ in respect to temperature effects. The experiments of the Lapicques and of Storm van Leeuwen and van der Made suggest that all reflexes have an optimum temperature, but it is possible that responses to different types of stimulation may have different optimal temperatures.

TEMPERATURE CONTROL. Evidence was advanced by Barbour (1912), confirmed by Hashimoto (1915) and Prince and Hahn (1918), that temperature control was achieved by reactions to changes of temperature of the central nervous system itself. In these experiments, where the region of the corpus striatum was warmed or cooled through a metal tube previously introduced, the actual temperature center probably lay at some considerable distance from the tube, for quite high temperature differences (at least 4° or 5°C.) had to be used to obtain reactions. With such temperatures some reflex stimulation of cranial nerves may have occurred, but is unlikely to have been significant. On the other

hand Cloetta and Waser (1914) were unable to obtain responses such as those Barbour described, when they heated the brain by diathermy, even though a rise of 1° or more might be recorded by a thermocouple in the lateral ventricle. The actual position of the center or centers controlling body temperature is probably in the thalamus, hypothalamus, or tuber cinereum, though certainly above the level of the pons and medulla (Isenschmid and Krehl, 1912; Leschke, 1913; Isenschmid and Schnitzler, 1914; deBarenne, 1919; Rogers, 1919; Bazett and Penfield, 1922).

Recent evidence, which is very strongly in favor of a control principally through the actual temperature of the blood supplying the temperature center, has been advanced by Sherrington (1924); in his experiments dogs rendered completely insensitive, except for the head and neck, by high spinal transection, shivered anterior to the lesion when the hind part of the body was immersed in cold water within a hot room, although the cooled skin was completely insensitive. Though, as he points out, an explanation of the results is possible on a reflex basis, if Ebbecke's hypothesis of the mode of origin of temperature sensations be accepted, yet, when considered with other evidence, a more probable cause would appear to be the direct effect of temperature changes on the central nervous system.

There is also abundant evidence that the temperature of the center itself cannot be the only factor in temperature control. Luchsinger (1877a) noted that sweating might be readily induced by substances such as pepper applied to the surface of the tongue, and recently Stary (1925) has described a fall of rectal temperature in rabbits after introduction of pepper extracts into the stomach. Liljestrand and Magnus (1923) have shown in themselves that the rectal temperature fell considerably in a bath containing CO_2, but that the subject showed no reaction to this fall of temperature and even had a subjective sensation of warmth. This subjective sensation of warmth from CO_2 is noted by Winternitz (1902) as the result of carbon dioxide gas applied to the skin and by Pembrey (1913) from breathing high carbon dioxide percentages by mouth. Goldscheider and Ehrmann (1924) found that carbon dioxide, and to a less extent oxygen, applied to the skin intensified sensations of warmth. Strasser (1923) noted that sweating may be induced in baths without a rise of rectal temperature, and both he and Bazett (1924) observed that shivering might be induced after a hot bath while the rectal temperature was as high as 38.9°. Sweating may be induced by exercise before any appreciable rise has occurred in rectal temperature (Berner and co-workers, 1925). With radiant heat hyperpnea may be induced without any rise in rectal temperature (Kroetz, 1914).

On the other hand these phenomena need not necessarily depend on simple peripheral reflexes. The observations of Stewart and Walker (1913) of reflexes from skin devoid of temperature sensations have already been mentioned (p. 569). L. Hill (1921) found that the sweating induced by a hot room could be prevented by the insertion of one arm in cold water and that this preventive effect disappeared if the circulation through the arm was stopped, even though the sensations of cold persisted; such a result might depend on deeply situated end-organs, or on temperature changes in the central nervous system. Goldschmidt and Light (1925b) found that their subjects had no tendency to shiver while the hand and forearm were immersed in very cold water, but that on withdrawal, when the circulation again became freer, a sensation of cold (usually associated with shivering) was often noted in the region of the axilla. Reflex effects from hypothetical deeply situated temperature end-organs (such as those considered by Meyer, 1920) cannot be definitely excluded.

The importance of both the temperature of the central nervous system and of skin reflexes in temperature control has been particularly advanced by Richet (1898); O'Connor (1916, 1919) has also brought forward evidence of such a dual control, both in regard to the metabolic and vasomotor responses. As described by Richet the control is a reflex phenomenon with the temperature of the center playing an important part only after a considerable change has occurred, and O'Connor also interpreted his experiments as indicating two separate and distinct mechanisms. Such an hypothesis seems unlikely and has not conclusive experimental support.

An alternative theory, which may perhaps be suggested tentatively, would be to suppose that the control is exerted entirely reflexly, sensory impulses of cold inducing one type of response and those of warmth the other; the temperature center would then under ordinary circumstances integrate these conflicting impressions and such a view would be in agreement with our subjective sensations, for we may occasionally have a general sensation of warmth, to which we may react reflexly, while conscious that some parts are abnormally cold (e.g., the hands or feet). Such an integration of peripheral sensations could not readily bring about the observed control of the central body temperature, but if a fall in temperature of the central nervous system tended to exaggerate reflex responses to cold (compare Lapicque's experiments, p. 581), while a rise in temperature causes an exaggeration of reflex responses to warmth, if, in fact, the optimum temperatures in the central nervous

system for the two responses were not identical, a temperature control could be achieved, The sensory impulses concerned would certainly include conscious exteroceptive impressions, and possibly also subconscious proprioceptive impressions from deeper tissues; local changes due directly to alterations in temperature might be utilized to assist in the active response. Such a system would readily explain various anomalies, such as the irregularities of metabolism in Löwy's experiments (1889), the fall of body temperature in baths containing CO_2 (Liljestrand and Magnus, 1922), or the shivering of a subject when the rectal temperature is about 39° (Strasser, 1923; Bazett, 1924). The existence of two different optimal temperatures for different reflexes is not impossible, especially since in cold-blooded animals even the same reflex may have two different optima (Storm van Leeuwen and van der Made, 1916), and since it can readily be explained on theoretical grounds, if in some part of its course the frequency of the nervous impulse associated with a cold sensation differed from that associated with warmth.

Such a tentative hypothesis attempts to incorporate in the older theories, such as those of Richet (1898) and of Meyer (1920), the principles elucidated by Sir Charles Sherrington, to whom I owe much. I shall, however, only have been justified in expressing it here in such a definite form, unsupported by experimental evidence as it is, if it succeeds in stimulating research, which may prove or disprove it.

Symptoms and acclimatization to temperature. Exposure to high temperatures sufficient to cause a rise of body temperature is usually accompanied by symptoms induced by the hyperpnea and vascular dilatation; the former of these is particularly in evidence the more rapid the temperature rise, and the discomfort seems due to an inability of the organism to adapt the acid-base equilibrium to the new conditions, although the opposite change from a high to a normal body temperature can apparently be made as rapidly as desired with no symptoms in spite of the induction of a somewhat subnormal ventilation rate (Bazett, 1924; Landis and co-workers, 1925). The dehydration which results from sweating may cause symptoms of collapse (Adolph, 1924; Bazett, 1924), which were observed in the case of one subject after a loss by sweating of some 3 to 4 per cent of the body weight. Under such circumstances an extreme restlessness may be noted, which is relieved by raising the lower limbs, and which appears similar to that seen after hemorrhage or in surgical shock, when venous return to the heart is subnormal. The muscular movements may, perhaps, be regarded as a reflex tending to assist venous return.

Vernon (1923) experimenting with muscular work under warm room conditions found considerable evidence of acclimatization. Conditions which at first produced considerable changes in pulse rate later produced much less change, and the same was true of the effect on respiratory rate. He pointed out that Sutton's figures (1909) showed less increase in metabolism with similar body temperature changes in his later experiments. A respiratory acclimatization was noted by myself (1924), even though these experiments were spread over a long period of time, and circulatory adaptation has also been noted in my laboratory in some unpublished experiments made by a group of students. Six students remained stimultaneously for one hour in a room saturated with moisture and kept between 35° and 37°C. Of these subjects all showed some increase in pulse rate, but in two subjects in the earlier experiments both the systolic and diastolic blood pressure fell considerably, in one subject they both rose, while in the other three the changes were slight and indefinite. After the experiments had been repeated 10 to 12 times, the blood pressure changes were gradually reduced and had become negligible in all subjects, though the pulse rate changes were still present.

The acclimatization of natives to their own climatic conditions is well recognized. This may be noted even among the varying climates of one country; thus the high room temperatures common in American houses, and often criticized, merely reproduce the ordinary "comfortable" summer temperature of the locality and are in consequence higher in the southern than in the more northern states. The tendency seems to be to maintain conditions as uniform as possible. Similarly the cold rooms in England merely reproduce the ordinary average summer temperature of that country. The capacity of the individual to adapt himself to such conditions is, however, harder to understand; it certainly cannot be accounted for on a basis of the degree of fat covering (which is less perhaps, rather than greater, in England than in America), but must rather depend on a metabolic or circulatory adaptation. It has been noted by both Knipping (1923) and Eijkmann (1924) that those acclimatized to the tropics sweat to a much less extent than do the newcomers, so that they do not have a wasteful dehydration from fluid which drips off, though possibly they evaporate as much water as do the others. Comparison of Americans from southern with those from northern states readily confirms such statements. On the other hand, in hot room experiments Borchardt (1926) found that those subjects who did not withstand the conditions well, and who readily showed a hyperthermia, were those who reacted with sweating of a low grade.

Muscle cramps, consisting of tonic and clonic contractions, are well recognized as an effect of exposure to heat for a long period, as in such occupations as stoking. They are commonly accompanied by headache, lassitude, vomiting and diarrhea, and may occur even when the body temperature is almost normal (Schmidt, 1901). The symptoms are particularly liable to occur in subjects who have drunk large quantities of water (Madsen, 1920). The symptoms observed are very similar to those described by Rowntree (1922) as the result of water intoxication. Moss (1923) found that miners working under excessively warm conditions consumed more salt with their food than did other individuals and that the more acclimatized workers had a higher rate of sweat production than did the others. He found that the symptoms of cramps were less liable to occur if the miners drank saline (about 0.2 per cent) instead of water, and noted that once a miner had experimented with drinking saline, he preferred it to water. It seems probable that such symptoms are caused by abnormal proportions of salts and water in the blood or tissues.

High temperatures are apt to cause a hyperemia of the conjunctiva, which may progress to a mild inflammatory reaction. McConnell and Houghton (1923) found that this was reproducible by the instillation into the conjunctival sac of a small quantity of sweat; they therefore considered that this reaction is caused simply by sweat running into the eyes. This however would not account for the occurrence of a similar conjunctivitis in rabbits in Heyman's experiments (1919), where the blood was warmed by being passed through a cannula joining the carotid artery and jugular vein. Hence, this reaction has not yet received an adequate explanation.

Conclusion. No attempt will be made to summarize the data and theories advanced in this paper, but it may be well to enter a plea for the more careful consideration of the effects of local changes in temperature on physiological processes in the mammal, and to reiterate a warning against the too prevalent assumption that the temperature recorded within the trunk may be supposed to exist throughout the whole organism. I should also like to take this opportunity of thanking my colleagues in my department, with whom the various reactions have been discussed, and who have often given me very valuable assistance.

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OVARIAN AND PLACENTAL HORMONES

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The ovaries, although primarily concerned with the genesis of ova, control the development and function of the female sex organs and the determination of secondary sex characters. That this control is accomplished by hormonal action has been clearly demonstrated by the preparation of ovarian extracts which upon injection into ovariectomized animals induce certain of the physiological reactions normally dependent upon ovarian endocrine function. Certain other aspects of this endocrine control are still obscure, but a basis for further study has been laid by the demonstration of at least one essential ovarian hormone. An identical or very similar substance can be extracted by the same methods from placental tissues of certain species. As yet it has not been possible to distinguish between purified material from these two sources by either chemical or physiological reactions. It is possible that there are other hormones in either the ovary or placenta but this seems still to be an open question.

The evidence concerning ovarian and placental hormones has been accumulated by means of several methods which have utilized different viewpoints.[1]

FUNDAMENTAL POINTS IN THE NORMAL ANATOMY AND PHYSIOLOGY OF THE GENITAL ORGANS OF FEMALE MAMMALS. The evidence grouped under this heading consists of the correlation of structural changes in the genital organs at different phases of the sex life of female animals. These studies have established the normal range of events in a considerable number of species.[2] Conclusions drawn from such studies

[1] Since in the course of collaboration upon work in this field the writers have specialized in certain phases of the experiments, the present review will be undertaken in the same way; responsibility for statements concerning animal reactions will be borne by Allen, that for biochemistry by Doisy.

[2] The following animals have been extensively studied and the following investigators should be cited among important contributors by this method:

Rabbit.....Heape (1900), Ancel and Bouin (1909), Hammond and Marshall (1914, 1925).

have resulted in the formulation of far-reaching hypotheses which have served as necessary foundations for the experimental attack of the problem. Therefore, besides defining the functional possibilities, these studies have provided that most necessary tool of experimental approach in this field,—the range of normal animal reactions used as biological tests for the presence of hormones.

The earlier contributions among those listed in footnote 2 have recently been reviewed by Marshall (152) (153), Corner (42), and others. Detailed discussion of the above mentioned investigations with specific citations cannot be undertaken in this review. A brief sketch of certain essential points of these studies of normal conditions in female mammals would stress at least the following points.

The prenatal and prepubertal periods. During the prenatal period it seems probable that the differentiating gonads determine the development of the other genital organs. From the time of birth to the attainment of sexual maturity a further progressive development of the genital organs occurs and certain distinctive secondary sex characters develop, apparently, because of ovarian endocrine influence.

During these two first periods of life of female mammals the ovaries are constituted of two distinctive tissues;—partly developing follicles and certain other "interstitial cells," epithelioid in character lying between the follicles. Considerable numbers of follicles at different stages of development are always present. Interstitial tissue seems to vary greatly in amount in ovaries of different species.

The adult non-pregnant period. The sexually mature period of female life opens with an acceleration of growth of the genital tract and mammary glands. In the lower mammals this rapid growth culminates in a period of oestrus or heightened sexual activity. If the animal is not mated a period of retrogression follows. These two phases of growth and retrogression in the genital tract and mammary glands recur in the absence of pregnancy at rather regular intervals during the sexually mature life of the animal.

Guinea pig.....Loeb (1909, 1911), Stockard and Papanicolaou (1917).
Dog.....Marshall and Jolly (1905), Keller (1909).
Mouse.....Sobotta (1895, Kirkham (1907, 1910), Allen (1922), Parkes (1926).
Ferret.....Robinson (1918).
Rat.....Long and Evans (1922).
Dasyurus.....Hill and O'Donoghue (1913).
Opossum.....Hartman (1916, 1919, 1923).
Pig.....Lewis (1911), Corner (1917, 1921), McKenzie (1926).
Cow.....Murphey and others (1926).
Monkey.....Heape (1897, 1900), Van Herwerden (1905), Corner (1923).
Man.....Hitschmann and Adler (1908), Meyer and Ruge (1913), Shroeder (1915), and many others.

Since rodents have been extensively used as test animals for sex hormone studies, a more detailed consideration of the changes in the genital organs during the oestrous cycle will be undertaken. It was difficult prior to 1917 to determine accurately the stage of the oestrous cycle of guinea pigs, rats and mice from external observations. Consequently certain details of normal conditions in non-pregnant female rodents had remained obscure until recent times. In 1917 Stockard and Papanicolaou (202) showed that the cycle in the guinea pig could be followed in the living animal by microscopic examination of the cell contents of the vaginal lumen which changed characteristically during the 16 day cycle. This method made possible the thorough study of the oestrous changes in this animal. It was later applied to the study of conditions in the rat by Long and Evans (150), in the mouse by Allen (2), Parkes (168) and in the opossum by Hartman (93). In these animals there is a definite progression of types of cells in the vaginal lumen which depends upon periodic growth of the vaginal epithelium to the point of formation of a cornified stratum. This is followed by a sloughing of the superficial layers and an infiltration of the epithelium by leucocytes. The growth changes in the vagina of the rat and mouse are paralleled by growth and secretion in the uterus which is in turn followed by degeneration and leucocytic infiltration. Waves of growth and regression are also present in the uterine tubes and mammary glands.

Ovarian function is correlated with these cyclic changes for growing follicles are present in the ovaries while pro-oestrous growth is going on in the other genital organs. These follicles may rupture and extrude their ova toward the end of the oestrous period or they may fail to ovulate and undergo atresia. Ovulation may be spontaneous as in the guinea pig, rat and mouse but may require the added stimulus of mating as in the rabbit and ferret. If ovulation does occur additional structures, the corpora lutea, develop in the ruptured ovarian follicles. In animals such as the rabbit, guinea pig, dog, monkey and man, ovulation with corpus luteum formation seems to be necessary for full growth and secretion of the endometrium.

The corpora lutea although derived primarily from hypertrophied follicular tissue are distinguished by deposits of lipoid material. These deposits have been considered sufficient to set the corpora apart as histologically distinct structures, but it should be remembered that in most mammals they are composed chiefly of the former granulosa cells of the walls of ruptured follicles. For this reason, during the period immediately following ovulation a transition structure, the early corpus luteum, might well be distinguished from the fully formed corpus luteum.

If ovulation does not occur, and there is much evidence that it often does not occur during many apparently normal oestrous and menstrual cycles even in "spontaneously ovulating" animals (mouse, monkey), the large follicles undergo atresia and resorption. Under such conditions certain of the non-pregnant cyclic changes of the genital organs transpire without the formation of corpora in the ovaries. In animals in which a mating stimulus is necessary for ovulation, an extended period of oestrus may be correlated with the longer persistence of large or cystic follicles in the absence of mating.

The primates have a non-pregnant sexual cycle which differs in several respects from the oestrous cycles of the lower mammals, the outstanding difference being

the occurrence of menstrual hemorrhage from the uterus. Another difference is that the menstrual cycle has no restricted mating period as in the oestrous cycle, the sexual instincts being diffused over a large part of the intermenstrum. The evidence indicates that follicles usually develop during the first two weeks of the cycle dated from the onset of the previous menses. Consequently ovulation occurs most frequently toward the end of the second week. But during the third week and possibly even later, growth and secretion may continue in the genital tract. This latter phase seems comparable to the pseudo-pregnant period described for the rabbit, dog, opossum and other mammals. Such a period of growth and secretion continued after ovulation is normally either absent from or much shortened in the brief oestrous cycles of some of the rodents.

From these differences in oestrous and menstrual cycles a difference might be expected in the ovarian influences controlling these functions. One point, however, seems definitely established for both types of non-pregnant cycle; namely, apparently normal cyclic function (as observed externally) may be maintained without development of follicles to the point of ovulation and corpus luteum formation in the non-pregnant animal. This is not entirely in harmony with the present day emphasis on the necessity of a growth producing hormone from the corpus luteum as an essential factor in the mechanism of oestrous and menstrual phenomena.

The reproductive cycle. The reproductive cycle as distinguished from the non-pregnant sexual cycle might logically begin with mating reactions. Normally the lower mammals show evidences of interest in sex only when the female is in oestrus, at which time large follicles are usually present in the ovaries. Ovulation and insemination therefore follow coitus. In some of the rodents with short cycles certain transient retrogressive changes may appear in the vagina and uterus while the segmenting ova are in transit in the tubes. In mammals having longer cycles such changes are less clearly indicated during this time. In both types a period of continued growth in the uterus and mammary glands follows implantation of the fertilized ovum, while in the ovaries there is a suspension of advanced follicular growth and a development of corpora lutea. A similar condition of pseudo-pregnancy can be induced by infertile copulation with vasectomized males or by cervical stimulation.

The development of relations between mother and embryo is too involved to be considered at length in this paper, but certain aspects of this phase of reproduction are essential to a consideration of placental hormones. As the gestation period advances growth of the uterus and mammary glands continues. With the development of the embryonic membranes, especially the differentiation of the chorion, several different structural types of maternal-embryonic relations are developed in different species of mammals: a, the embryo may lie in the lumen of the uterus with a poorly villated chorion in close apposition to an intact maternal mucosa as in the pig; b, the chorion may be covered with quite well developed villi distributed diffusely all over its surface, as in the horse, or c, these villi may be grouped in patches as in the cotyledonary arrangement in the cow and sheep; d, in carnivors the placenta becomes a zonular structure or belt around the middle of an elongated chorionic sac; e, in rodents and man it assumes a discoid form.

In the ungulates it is possible to separate the embryonic from the maternal part and make separate biological analyses of the hormone. In the human pla-

centa the maternal and embryonic parts are too intimately connected (with complete destruction of intervening uterine epithelium) to allow such a mechanical separation. These structural modifications in tissues concerned in maternal-embryonic relations suggest possible differences in the endocrine functions of placental structures of different types.

Sexual senescence. The close of the period of sexual maturity is indicated by a retardation of sex function and the beginning of atrophy of the genital organs. The transition may be gradual. It may begin by the omission or irregularity of oestrous or of menstrual periods and end by their complete disappearance. Examination of the ovaries at this phase of life usually shows the absence of large follicles and recently formed corpora lutea. There is also a decrease in the number of follicles and eventually it may be difficult to find ova, the ovaries becoming small masses of fibrous tissue. This phase of sex life might be compared to a gradual ovariectomy and emphasizes again the dependence of the other genital organs upon the endocrine function of the ovary.

Hypotheses concerning causes of cyclic sex phenomena. From a correlation of the condition of ovarian structures with the changes in the other sexual organs several hypotheses have been formulated as to their possible interrelations.

During the prenatal and prepubertal periods interstitial tissue has been stressed as a source of production of hormone. The importance placed upon the endocrine function of the interstitial cells of the testis has by analogy tended to emphasize ovarian interstitial tissue, "the puberty gland" of Steinach, as a seat of hormone production. The development of secondary sex characters is supposedly dependent upon this tissue.

When the animal becomes sexually mature and large follicles are found in the ovary at oestrus one inference is that these large follicles are a source of hormone. In case ovulation occurred during the cycle in question, corpora lutea formed in the ruptured follicles have been considered an additional source. From anatomical studies of the ovaries of most mammals the corpora lutea loom large as important structures. In the first place they usually attain a larger size than the follicles in which they began development and in many cases they protrude from the surface of the ovary in a prominent manner. They have been vascularized and consequently show a red surface as compared to the pearly translucent wall of the large follicles. Furthermore they persist for a longer period of time than do the large follicles. In the pig, for instance, which is the principal source of ovarian preparations, follicles of medium and large size are present for only a few days. Corpora resulting from the ovulation of such follicles may persist in a prominent condition for from two to three weeks. The development of

lipoid deposits in this luteal tissue adds further to their prominent appearance during later development and seems to be associated with their characteristic yellow color. Some investigators have considered these lipoid accumulations to be indicative of hormonal secretion. The possible endocrine function of the corpus luteum has been emphasized by its prominence and by its development parallel with the uterine changes of the premenstrual period in primates.

When ovulation does occur and successful fertilization is obtained the corpora lutea remain large and active throughout pregnancy. This extended period of function led to the hypothesis that the corpus luteum secreted a hormone which sensitized the mucosa of the uterus to prepare it for the implantation of the fertilized ova. Loeb (135) has shown that the uterus of the guinea pig will react to artificial stimulation by the formation of decidual tissue at times when large corpora are present in the ovaries. It has also been proposed that the corpus luteum plays a part in exerting growth influences upon the mammary glands during gestation and that it controls or retards follicular development by the secretion of an inhibitory hormone.

Marshall (152, p. 368) concisely summarizes Fraenkel's theory (69) as follows:

> The corpus luteum is a ductless gland which is renewed every four weeks during reproductive life in the human female, and at different intervals in the various lower mammals. Strictly speaking, there is only one corpus luteum which represents the ovarian organ of internal secretion, and is regenerated periodically in slightly different positions in the ovaries. Its function is to control the nutrition of the uterus from puberty until the menopause, to prevent it from lapsing into the infantile condition or undergoing atrophy, and to prepare its mucous membrane for the maintenance of the ovum. If the ovum be fertilized, the corpus luteum is responsible for maintaining the raised nutrition of the uterus during the first part of gestation. If the ovum be unfertilized it merely produces the hyperaemia of menstruation, and then undergoes degeneration until it is renewed in a fresh position. Since the corpus luteum is, *par excellence*, the ovarian gland, "lutein" or the extract of this organ, and not preparations of the entire ovary, should be employed for the purpose of ovarian medication.

In Marshall's opinion (153, p. 339),

> although the original part of Fraenkel's theory is now generally accepted, it is not justifiable to regard the corpus luteum as the responsible factor in the attachment and nutrition of the ovum or early embryo in any other sense than that implied in the statement that this organ, through the secretion it produces, acts as a stimulus to the uterine mucosa and the maintenance of the increased uterine nutrition which are necessary for the occurrence of gestation.

The emphasis placed by Fraenkel's work upon the endocrine function of the corpus luteum has profoundly influenced subsequent work on ovarian hormones.

With the progress of gestation the development of placental structures introduces a new complication into the endocrine problem. At least three possibilities might explain the endocrine function of the placenta: 1, that the placenta produces a uterine and mammary growth hormone, or 2, that it is in some way related to such a substance produced in the ovary, and 3, that it secretes an inhibitory hormone affecting lactation which is removed at parturition. It is of course difficult to consider normal function of the placenta apart from the embryo or fetus.

During the period of sexual senescence ovulation probably occurs rarely. Consequently there are no recent corpora lutea in the ovaries. This has been considered additional evidence for endocrine function of the corpus luteum during sexual maturity. But the absence of corpora must also mean the absence of follicles which have attained to ovulation size. This evidence might then apply with equal force to large follicles.

To summarize briefly concerning hypotheses of secretion of ovarian hormones: before the attainment of puberty both immature follicles and interstitial tissue are possible sources of female sex hormones. After puberty the corpus luteum might be added as a third possible source. An additional factor during the reproductive cycle is introduced by the formation of the fetal membranes and placenta. The placenta (or the chorion) is in some way related to the endocrine function of the ovaries during gestation.

THE EXPERIMENTAL ATTACK OF THE PROBLEM OF HORMONAL CONTROL. *Excision and transplantation of parts of the genital system.* The many hypotheses formulated from morphological studies have already been quite widely tested by experimental attack of the problems involved. It has been well established by many experiments that removal of the ovaries from the immature animal causes a cessation of development in both the primary sex organs and secondary sex characters (152). Excision of the uterus and of the mammary glands has apparently failed to establish any definite endocrine effect upon the ovaries. The castration experiments, however, have definitely proved that the ovary is the source of immediate control of sexual functions. The ovariectomy experiments still left open the possibility that such control was effected through the nervous system.

The problem was further approached through transplantation of ovaries from their natural sites to other parts of the body of the same individual. Such ovaries as became vascularized functioned in exerting their normal endocrine influence. Therefore ovarian control is probably effected by hormonal means through the blood stream. Knauer (118) and Halban (90) were among the first to submit conclusive evidence on this point. The recent exhaustive experiments by Long and Evans (150) leave no doubt concerning the endocrine function of ovarian transplants in mammals. In some of these transplantation experiments follicles developed to full size and in many cases corpora lutea were present. Consequently no conclusive evidence was presented concerning the part played by any particular ovarian tissue.

The reader is referred to Lipschütz (133) for a recent review of evidence from ovariectomy and gonad transplantation experiments.

X-ray effects. By use of the selective action of x-rays it has been shown that follicular tissue of the ovary can be destroyed and still leave the ovary with enough endocrine power to prevent atrophy of the uterus and mammary glands. Such x-rayed ovaries show relatively large amounts of tissue somewhat similar to "interstitial tissue." Bouin, Ancel and Villemin (25) and later Steinach and Holzknecht (201) and others have presented evidence upon these points. As far as the writers are able to judge, these earlier experiments with x-rays brought forth no evidence to indicate that the rayed ovaries retained the power of inducing cyclic changes in the other genital organs. Recently, however, Parkes and his collaborators (169) (170) (171) have shown that critical doses of x-rays may quite completely destroy follicular tissue in the mouse but still leave the ovaries with the capacity of producing enough hormone to cause apparently typical oestrous changes in the vagina and uterus.

Steinach and Holzknecht emphasized ovarian interstitial tissue and called it the "puberty gland." They believed that its secretion is responsible for the attainment of puberty including the development of the secondary sex characters. The extent to which interstitial tissue shares this function in the normal animal is still an open question, but apparently the experiments of Parkes demonstrate that ovarian tissue after having completely lost its oögenic potentialities may retain its hormonal function. For further references to the literature upon normal cyclic changes, castration and transplantation experiments, and x-ray effects the reader is referred to Marshall (152) (153), Carlson (38), Novak (159), Evans (56), Corner (42), Lipschütz (133) and Biedl (23).

A FEW QUOTATIONS FROM EARLIER REVIEWS. Before beginning to review the evidence from injection experiments a few opinions expressed in other discussions of this subject between 1917 and 1924 are of interest.

Marshall (153, p. 349) in summarizing writes,

> It is not known what precise ovarian elements are responsible for the production of the hormone which is an excitant for the development of the female organs and characters or the possible inhibition of the distinctly male ones. . . . .

Later on page 364 (152),

> Lastly it must not be forgotten that, whereas it is exceedingly probable that the pro-oestrous changes of the uterus are brought about by the specific excitant or hormone arising in the ovaries, little or nothing is known concerning the chain of causation leading to that disturbed state of the nervous mechanism, the existence of which during oestrus is so plainly manifested in the display of sex feeling.

After discussing the status of the ovarian elements capable of possessing internal secretory functions, Marshall writes,

> The conclusion clearly to be drawn from all these experiments and observations is that the ovarian hormone which produces oestrus or heat is different from that which is responsible for maintaining normal uterine nutrition.

Corner (42, p. 471) after reviewing the work done on normal animals summarizes the present status of our knowledge as late as 1922:

> Finally it will be seen that our information is chiefly in the nature of a chronological correlation between the outward events of the cycle, the ovarian and uterine changes, and the movement and implantation of the ova. The actual causal relations, endocrinal or otherwise, between the follicle and corpus luteum on one hand and the uterus on the other, are largely unexplored.

Carlson (38) in discussing the earlier work writes,

> The attempts to elucidate the ovarian hormones by the administration to animals of crude or purified extracts of the ovary or its different anatomic constituents have been neither systematic nor extensive. As a result this work to date is inconclusive and essentially negative.
>
> None of the ovarian hormones have so far been isolated, as determined by reliable biological or chemical tests. And it is still an open question whether in the experimental animals any of the ovarian functions can be maintained by substitution therapy; that is, by feeding or injection of ovarian extracts.

Lipschütz (133, p. 32) writes,

> The impression one gets when reading the contradictory results obtained by different authors with extracts of testes, ovaries, placenta and fetus is not a favorable one.

and later

> It must also be taken into consideration that it is not possible to imitate by injection the real quantitative and time relations of the normal glandular function, to imitate, so to speak, the rhythm of normal functions. . . . .

and later

> Quantitative and time relations in internal secretions have so far been very insufficiently studied on an experimental basis.

A careful scrutiny of these recent reviews impresses the reader with the skepticism of the authors and indicates the slow acceptance of the results obtained in the earlier work with ovarian extracts. This may have been due partly to certain difficulties inherent in the nature of the test animal used. The rabbit was used by the majority of investigators in the earlier work with ovarian extracts. The recent book, *Reproduction in the Rabbit*, by Hammond (91), shows quite clearly that the full range of normal sexual function had not previously been carefully worked out in this animal. The best test used for activity of extracts was the reaction of the uterus to injections. This test is compared on page 616 with the later vaginal test in rats and mice which has greatly simplified the study of these problems.

BASIC EVIDENCE OF HORMONAL CONTROL FROM ADMINISTRATION OF EXTRACTS. It was planned at first to restrict this review to the evidence from injection experiments, but since evidence from this source represents only one of several worthwhile viewpoints and since it is so dependent upon the studies of conditions in normal animals it seemed desirable to include the foregoing sketch as a foundation. The review proper deals mainly with direct evidence obtained by the use of extracts.

In a recent volume of the *Handbuch der normalen und pathologischen Physiologie*, A. Biedl (23) has given a very careful and thorough review of the evidence regarding the internal secretion of the gonads. His paper deals chronologically with the work of investigators in this field and describes the contributions in detail. The present review will deal with the evidence under subject headings rather than following the chronological order of the numerous contributions.

Early work As a beginning toward administration of ovarian preparations the early attempts to alleviate the symptoms of natural and operative menopause may be cited. Influenced by Baumann's work on the thyroid (22), Landau administered dried ovarian preparations per os (121). Due to the low percentage of successful results from this treatment Landau warned against complete ovariectomy.

Among early experiments upon laboratory animals were the attempts of Jentzner and Beuttner (112) and later Bucura (34) who sought to prevent atrophy of the uterus of rabbits following ovariectomy. They used saline extracts and dry residues of ovaries, but did not obtain promising results.

Real advances in the demonstration of effects of ovarian extracts begin with the positive results obtained by Adler (1), Fellner (61), Schickele (183, 184), Aschner (19), (20), Iscovesco (110), Okintschitz (162), Herrmann (99) (100), Seitz, Wintz and Fingerhut (192) and Frank and Rosenbloom (81)

Animals used. The animals used for studies of ovarian preparations fall logically into two groups: 1, those which have served as sources of material for preparations of hormone; and 2, those into which injections were made in attempts to obtain specific hormonal effects. The first group, the source of supply of ovarian material, for obvious reasons consists mainly of the larger animals, the pig, cow, sheep, horse and man. The second group, the test animals, is composed for the most part of the smaller "laboratory" animals, the dog, rabbit, guinea pig, rat and mouse.

Localization of the function of hormone production. The material used for preparations of extracts has included a wide range of tissues but ovaries and placental tissues seem to be the only well established sources of active substance. Whole ovaries have been used in many instances. Attempts have also been made at localization by selecting the ovaries used for extraction with regard to follicle, corpus luteum or interstitial cell content. This selection by early investigators seems to have been greatly influenced by Fraenkel's important work which emphasized the function of the corpus luteum (page 605), for a large part of the best work prior to 1920 stressed the corpus luteum as the principal ovarian source of active material (1), (61), (100), (81). Either whole ovaries from pregnant animals containing small follicles and well-developed corpora were selected or the corpora were clipped from the ovary and extracted separately. In the more recent work large follicles have been emphasized as the principal ovarian source of the hormone and their contents isolated for use in a fresh condition or for the preparation of extracts. Attempts have also been made to isolate interstitial tissue by testing residual ovarian tissue after removal of corpora lutea and the contents of large follicles. Obviously many small follicles are inevitably retained in such "residual tissue." Also unless the granulosa cell walls of the large follicles (from which the fluid may have been removed) are carefully dissected out, the "residual tissue" still contains much follicular material.

In considering a separation of the ovary into its constituent tissues for the study of possible localization of hormone production, it seems important that fresh ovaries be used. In some of the earlier tests corpus luteum substance which had been "degreased" was used. Other investigators have used frozen ovaries in which no adequate control tests have been made of possible post-mortem diffusion of ovarian tissue fluids. It would seem only a reasonable precaution that where conclusions are drawn as to localization of ovarian endocrine function, that the separation of tissues be made as soon after removal of the ovaries as possible.

Isolation of contents of large follicles by aspiration is relatively easy and less open to the possible criticism of contamination by tissue fluids from other ovarian sources. Previous to 1923, however, there appear only preliminary reports of positive results from injection of follicular fluid. These were by Sonnenberg (198), Wintz (209), Frank (71) and Seaborn and Champy (188). These will be considered more fully after a description of the animal reactions used as criteria of activity.

The writers have failed to find a successful preparation of extracts from isolated ovarian interstitial tissue. Residual ovarian tissue after removal of corpora lutea and of fluid from large follicles still contains many small follicles and (unless carefully dissected out) the walls of the large follicles from which the fluid was removed.

By far the most readily available source of placental extracts is the full term human placenta. With the placenta as well as with the ovaries attempts have been made to study the possible localization of production of active substance. The fusion of maternal and embryonic parts in the human placenta at full term is so complete that a separation into these component parts seems impossible. Early human chorionic vesicles, however, may be obtained comparatively free from maternal tissues. In the cotyledonary placentas of the cow and sheep a separation can be made, the principal branches of the chorionic villi being withdrawn from the maternal pits (8) (174). This process probably leaves some embryonic tissue in the "maternal pits" but makes a fairly thorough separation as far as the embryonic part is concerned. As yet no major distinction seems to have been established between hormone content of embryonic and maternal portions.

Substitution effects in ovariectomized animals. The most conclusive evidence for the action of ovarian hormones consists of substitution of extracts for the endocrine function of the ovaries. This requires the removal of the ovaries from the test animals. The response to ad-

ministration of extracts should be comparable to normal sexual phenomena.

Undoubtedly the most important criterion of activity of extracts used in the earlier work was the condition of the uterus (1) (183) (184) (61) (100) (162) (192) (81). The striking changes in this organ at different phases of reproductive life have focussed attention upon it from the beginning. The aim of some of the early injection experiments was merely to "maintain uterine nutrition" or prevent the atrophy of this organ which follows removal of the ovaries (112) (34) (110) (162). Later on, after the full range of cyclic variation in the uterus became more fully appreciated, comparisons of results from injections with the maximum oestrous, "predecidual" or even pregnant condition of normal animals were made (61) (100) (81). It is very difficult to evaluate these results. Review of the early experiments shows clearly that the atrophy following ovariectomy could be postponed and the uterus maintained in good functional condition (110) (162). Comparison with the ovariectomized control conditions was often striking. In several contributions uterine conditions apparently equivalent to those of normal oestrus are described and pictured (1) (61) (100). It is doubtful, however, if uterine conditions equal in development to pseudo-pregnant conditions have been obtained to date in ovariectomized animals by injections of extracts.

A second criterion of activity of injected extracts was the swelling and hyperemia of the regions surrounding the external genital organs (198) (61) (100). Such phenomena are prominent in rabbits, pigs, dogs and monkeys but slight in many of the rodents. In some instances the test animals were normal, either immature or in a sexually quiescent condition with ovaries still intact. Experiments attempting the induction of these phenomena by injections after removal of the ovaries were apparently successful in some cases. In other experiments, the number of injections given and the interval following injections during which observations of results were made, were not sufficient for development of maximum conditions (154) (198) (188). Many of these earlier results might be characterized as "transient signs of oestrus" (Marshall and Jolly, in the dog (154)). When considered alone this is far from satisfactory evidence of experimental oestrus.

A third criterion of positive results from ovarian and especially from placental extracts was the production of hypertrophy of the mammary glands (122) (19) (61) (100) (81). This effect was studied both in female and male test animals. Prominent nipples and considerable hyper-

trophy of mammary glands with growth of mammary ducts and alveoli have been recorded. Mammary growth has been demonstrated in the rabbit (61) (122) (100) and guinea pig (19). The opossum is an ideal animal for the study of this problem for the mammary glands are all crowded into a restricted area in the pouch. The hypertrophy of the gland in these animals can be accurately gauged by palpation (Hartman) (93). Marked mammary growth has been induced in ovariectomized opossums by injections of follicular and placental extracts (94). Reference to similar results in the monkey (9) will be made later.

These three specific criteria of activity served investigators previous to 1923. With four exceptions the ovarian extracts tested by these methods were made from whole ovaries or from isolated corpora lutea. Reports of tests of isolated follicular contents appear only as preliminary reports and with one possible exception they were made in normal test animals in which the ovaries were still intact. Consequently with this exception the results described from follicular fluid cannot be considered substitution effects.

Sonnenberg (1907) (198) briefly reported injections of small amounts of follicular fluid diluted with glycerin into normal rabbits. Only 1 cc. of diluted material was given to each animal and observations of results were limited to thirty minutes following the injection, a reaction time much too short to obtain a decisive physiological response. These results deal with transient hyperemia.

Wintz (1920) (209) reported a series of injections into rabbits. From later work it is clear that the number of injections given was insufficient to induce more than rudimentary oestrous conditions.

Frank (1922) (71) reported positive results (obtained in 1917) from the injection of liquor folliculi into normal rabbits. His report is quoted in full:

> A quantity of follicle fluid, obtained by puncturing ripening follicles from the ovaries of cows, was collected. In the one series the ovaries were derived from non-pregnant animals, in the second from those of pregnant ones. Two virgin rabbits were injected subcutaneously in each series with 1 and 2 cc. respectively, of the follicle fluid daily for ten doses. In all four animals, well marked hyperplasia of the uterus was noted, the material of the non-pregnant animals being the more effective.

Seaborn and Champy (1923) (188) injected liquor folliculi from the mare into two normal rabbits. This was followed in 8 to 10 hours by congestion of the genital organs and willingness to copulate.

The brief nature of these reports of effects of injections of liquor fol-

liculi accentuates the predominance of the idea that the corpus luteum was the principal ovarian source of hormone.

Vaginal reaction of rodents. A fourth criterion of activity of ovarian and placental extracts has recently been introduced (10) (14). Until relatively recent times the full range of cyclic changes in the vagina had not been appreciated. The description of conditions in the normal guinea pig by Stockard and Papanicolaou (202) has stimulated similar studies in the rat (150), and mouse (2), opossum (93), pig (158), cow (155), mare (187), monkey (40) and woman (132) (117). These studies have firmly established a cyclical acceleration of proliferation of the stratified epithelium of the vaginal wall, especially prominent in the rodents, which is correlated with the ovarian cycle.

One of the points of greatest practical value pointed out by Stockard and Papanicolaou was that this cyclical growth of the vaginal epithelium causes changes in the types of cells sloughed into the lumen. By correlating these cell types with the condition of the internal genital organs a new insight into the finer details of the oestrous cycle was obtained, for now it was possible to follow these changes in the living animal. With the use of this method the whole phase of reaction time of the oestrous cycle in rodents has been opened up. Previous to the introduction of this method the duration of the oestrous cycle in the mouse was estimated at about 17 days (Long and Smith, 151). Using this new method a cycle of 4 to 5 days has been established for both the rat (150) and the mouse (2) (168).

The work of Stockard and Papanicolaou awakened much interest in problems of sex. The study of the oestrous cycle in the normal mouse (2) was undertaken as a direct reaction to this stimulus. It seemed probable from conditions in the mouse that the ripening follicle alone might be responsible for the oestrous changes in the genital organs. The first tests of this hypothesis were surprisingly successful (3) (10). With a series of 3 to 6 injections of follicular fluid spaced at approximately 4 hour intervals into spayed rats and mice, typical oestrous changes in the vaginal cell types were induced. The reaction time of these experiments (48 hours) duplicated that of the growth phase of the normal cycle. This fourth criterion of activity of ovarian and placental extracts could be applied to the living test animal.

These results were checked by histological examinations of the internal genital organs (14). The vaginal wall had grown to full thickness and the typical oestrous cornified layer was present. The uterus was hyperemic—had grown and become greatly distended with secretion.

That the increase in size was due partly to growth by cell division was shown by the high incidence of cells in mitotic division indicative of extremely rapid hyperplasia. The extent of secretory activity could be gauged because in the rat and mouse this secretion is retained in the uterus. The fundamental reactions of the genital tract to this hormone were growth and secretion and these processes in the experimental animals were equal to the maximum occurring in the normal, non-pregnant animals as described for the rat (Long and Evans) and mouse (Allen). Secretion of the uterus of the mouse and rat might be considered a fifth criterion of activity of hormone.

Furthermore, ovariectomized animals brought into experimental oestrus by injections of follicular hormone mated normally and copulation was followed by the formation of typical vaginal plugs of coagulated ejaculate as in normal rodents. Ovariectomized rodents and normal rodents not in oestrus will not accept coitus. The mating reactions add a sixth criterion of hormonal action.

Following the last of the injections a degenerative phase set in which seemed equivalent both in morphology and reaction time to the normal.

It seems to us that this work demonstrated the following new points:

1. The isolated contents of the large follicles contain an active substance which alone is sufficient to induce maximum oestrous phenomena in rats and mice.

2. The essential features of the oestrous reaction are hormonal stimulation of the tissues of the genital tract to growth and secretion.

3. An absence or decrease of this hormone allows the degenerative phase of the cycle to set in.

4. The reaction time of the experimental cycle is equivalent to the normal cycle.

5. The female sexual reactions of mating also depend upon this active material from the large follicles.

6. A single hormone from the follicles is all that is essential to the mechanism of the non-pregnant cycle of the rat and mouse.

The vaginal reaction in the mouse and rat as a test for activity of extracts. Besides these conclusions concerning the endocrine mechanism of the oestrous cycle in the rat and mouse, the above experiments provided a very practicable test for activity of ovarian and placental extracts. This test is compared in table 1 with the uterine reaction of the rabbit used to a great extent in work upon ovarian and placental extracts previous to 1923.

With this simple, quick vaginal smear method of testing it has been

possible to extend both the qualitative and quantitative survey of sources of this hormone. It has also facilitated the chemical purification of ovarian and placental extracts.

SOURCES OF THE GENITAL GROWTH HORMONE. Our discussion of the sources of the hormone should be prefaced by the remark that our survey is based mainly upon physiological evidence. Though the physiological responses evoked by two crude extracts may be the same, it appears unwise to state that the two active principles are the same until they have been isolated as pure chemical substances and their

TABLE 1

A comparison of the uterine reaction of the rabbit with the vaginal reaction of the rat and mouse (as biological tests for activity of ovarian and placental extracts)

RABBIT TEST (UTERUS)	RAT OR MOUSE TEST (VAGINA)
1. Due to the internal location of the uterus effects of injections (requiring histological examination) were determined by a single observation at autopsy, necessitating a new animal for each test	1. By the technique of vaginal smears results can be observed at several intervals upon the living animal and the animal can be used repeatedly
2. Time relations of normal conditions were vague	2. Normal conditions were well established
3. The reaction time of the test was often a random quantity and usually required at least 4 to 10 days	3. A definite, shorter reaction time elapses equivalent to that of the growth phase of the normal cycle (2 to 3 days)
4. This test was not readily adaptable to quantitative use[3]	4. The vaginal test has a definite end point making its use as a quantitative unit possible
5. A larger amount of active material is required	5. The smaller amount required for a maximum reaction makes possible tests of smaller chemical fractions

[3] Asdell and Marshall (20) have shown that injections of follicular hormone into ovariectomized rabbits, although causing extensive uterine growth, do not include stratification or cornification of vaginal epithelium. Apparently then the vaginal smear reaction in the rabbit would be far from conclusive as a test of hormone action.

TABLE 2

Distribution of the ovarian hormone

SOURCE	RESULTS OF TEST	AUTHORITY	REMARKS
Ovaries:			
Cow.....	+	Adler (1912)	Aqueous extracts Uterine reaction
Hog.....	+	Iscovesco (1912)	Lipoid solvents Uterine reaction
Human.....	+	Allen, Pratt and Doisy (1925)	Vaginal reaction
Sheep.....	+	Allen and Doisy (1923), Parkes and Bellerby (1926)	Vaginal reaction
Hens.....	+	Allen, Whitsett, Hardy, Kneibert (1924)	Vaginal reaction
Horse.....	+	Parkes and Bellerby (1926)	Vaginal reaction
Corpora lutea:			
Cow.....	+	Adler (1912)	Confirmed by Fellner (1913), Herrmann (1915), Frank (1915), Fraenkel, S. (1923) Negative results or very low yields obtained by Allen and Doisy (1923) and Johnson and Gould (1925)
Hog.....	+	Iscovesco (1912)	
Human.....	+	Allen, Pratt and Doisy (1925)	
Liquor folliculi:			
Cattle.....	+	Frank (1922)	Uterine reaction
Cattle.....	+	Allen and Doisy (1923)	Vaginal reaction
Hog.....	+	Allen and Doisy (1923)	Vaginal reaction
Sheep.....	+	Allen and Doisy (1923)	Vaginal reaction
Human.....	+	Allen, Pratt and Doisy (1925)	Vaginal reaction
Mare.....	+	Hart, deJongh, Laqueur, Wijsenbeek (1925)	Vaginal reaction
Placenta:			
Human.....	+	Fellner (1913)	Confirmed by many investigators Uterine reaction
	+	Allen and Doisy (1924)	Vaginal reaction
Cow.....	+	Allen (1925)	Vaginal reaction
Sheep.....	+	Parkes (1927)	Vaginal reaction

TABLE 2—*Concluded*

SOURCE	RESULTS OF TEST	AUTHORITY	REMARKS
Blood:			
Women.....	+	Loewe (1925), Frank et al. (1925)	Vaginal reaction
Hogs.....	+	Frank (1925)	Vaginal reaction
Urine:			
Women.....	+	Loewe (1925)	Vaginal reaction
Liver.....	+	Schroder (1920);	Uterine reaction
	−	Lacquer (1925)	Vaginal reaction
Pancreas.....	−	Doisy, Ralls, Allen and Johnston (1924)	Vaginal reaction
Muscle.....	−	Parkes and Bellerby (1926)	Vaginal reaction
Testes.....	+	Fellner (1921); Laqueur (1927)	Uterine reaction; vaginal reaction
	−	Doisy, Ralls, Allen and Johnston (1924)	
Embryos:			
Pig.....	−	Doisy, Ralls, Allen and Johnston (1924)	Vaginal reaction
Eggs:			
Hens.....	+	Fellner (1925); (4 preparations)	Uterine reaction
	−	Doisy, Ralls, Allen and Johnston (1924)	Vaginal reaction
Fish.....	+	Fellner (1925)	Uterine reaction
	−	Doisy, Ralls, Allen and Johnston (1924)	Vaginal reaction

constitution and properties compared. In the case of the extracts of human placenta and cattle or hog ovaries, there is some chemical evidence that the active principles are similar if not identical. The evidence is based upon solubility and certain reactions such as oxidation, bromination, hydrogenation, hydrolysis, stability at high temperatures, etc.

The authors disclaim responsibility for the accuracy of the data of the preceding table which has been prepared by assembling information from a large number of papers. The sources of hormone are important from the biological and quantitative viewpoints.

The quantitative distribution of the hormone is important to the investigator who desires to obtain material for physiological studies and to the manufacturer of organo-therapeutic products. Since the

development of the more accurate assay method, it has been the custom to express the yield of hormone in mouse or rat units per kilogram of tissue. Data so obtained indicate that there are only two sources of supply worthy of consideration from a quantitative viewpoint, i.e., ovaries from the larger animals and human placenta. While we have not studied the problem sufficiently it has been our experience that the placenta usually contains somewhat more hormone than an equal weight of hog ovaries. More data are available on the relative yield of units from liquor folliculi and the residual ovarian tissue. Doisy, Ralls, Allen and Johnston (53) obtained from 220 to 2000 rat units per kilo of hog liquor folliculi from large follicles with the average value of later work (54) falling about 900. From ovaries from which the liquor folliculi had been removed, less than 200 units per kilo were obtained. Dickens, Dodds and Wright (52) and Parkes and Bellerby (175) do not agree with these findings, in that they do not find any more hormone in the liquor folliculi than in the residual tissue,[4] whereas the data of Laqueur and his associates are in agreement with Doisy's.

With respect to the corpus luteum, Frank (72) and his associates insist upon the presence of the hormone in that tissue but do not give any data on the units per kilo. Johnston and Gould (113) did not obtain positive tests with any of their extracts but the quantities used for injection do not rule out the possibility (one case excepted) of the presence of 30 or 40 rat units per kilo of corpora lutea. In a recent experiment Doisy (unpublished) obtained a negative test with the extract of 28 grams of mixed hog corpora but a positive test with the extract of 120 grams. Allen, Pratt and Doisy (16) have obtained positive results in a majority of tests of recent human corpora, the maximum obtained being 3700 rat units per kilo of tissue. These results are discussed and contrasted with quantitative yields from cow and pig corpora lutea.

Table 3 may prove useful in giving a clearer idea of the number of units obtained from the tissues studied by the various investigators.

METHODS OF PREPARATION. Since Brown-Sequard (33) suggested that the ovary produced an internal secretion, many attempts have been made to use ovarian preparations therapeutically (Landau, 1896) (121). Adler (1) refers to the unsuccessful effort of Regis (181) to alleviate ovarian disorders by the administration of ovarian products.

[4] Dickens, Dodds and Wright state that the follicles from which the fluid was obtained were not always large as ovaries were seldom obtained from animals in oestrus.

In 1912 Adler published a report upon the successful extraction of an ovarian hormone which was obtained from ovaries or corpora lutea. The aqueous liquid obtained by extraction of the hashed tissue with

TABLE 3

Amount of hormone obtained from genital tissues

TISSUE	YIELD PER KILO	OBSERVER
Liquor folliculi:		
Hog	878 R.U.	Ralls, Jordan and Doisy (1926) (Average of table 1)
Hog	600–1600 M.U.	Laqueur, Hart, deJongh and Wijsenbeek (1926)
Hog	34 M.U.	Parkes and Bellerby (1926)
Hog	167 R.U.	Dickens, Dodds and Wright (1925)
Cow	321 M.U.	Parkes and Bellerby (1926)
Mare	113 M.U.	Parkes and Bellerby (1926)
Human	433–7000 R.U.	Allen, Pratt and Doisy (1925)
Corpora lutea:		
Human	3700 R.U.	Allen, Pratt and Doisy (1925)
Hog	>8 but <25 R.U.	Doisy (unpublished)
Ovaries:		
Hog	219 M.U.	Parkes and Bellerby (1926) (a single experiment)
Hog	120 R.U.	Doisy, Ralls, Allen and Johnston (1924) (average of 3 tests)
Pig immature	220 M.U.	Parkes and Bellerby (1926) (average of 3 tests)
Cow	293 M.U.	Parkes and Bellerby (1926) (a single test)
Cow immature	211 M.U.	Parkes and Bellerby (1926) (average of 2 tests)
Sheep anoestrous	203 M.U.	Parkes and Bellerby (1 test)
Placenta:		
Human	400–700 R.U.	Doisy, Ralls, Allen and Johnston (1924)

R.U.—rat units.
M.U.—mouse units.
Exact relationship is not known but 1 R.U. is equal to at least 2 M.U.; probably > 4 M.U.

distilled water in the ice-box for 24 hours contained a substance which promoted growth and hyperemia of the uterus. The juice expressed

by a Buchner press at 450 atm. and diluted with saline was likewise active. Adler also reported that "ovarin" which apparently was a commercial preparation made by Professor Poehl of St. Petersburg, was a potent product.

Fellner (61) found that saline extracts of the placenta were more active than those made with water alone, but that both were less active than those in which alcohol or acetone was employed to extract the hormone. Aschner (19) likewise stated that the hyperemic agent was water soluble and Seitz, Wintz and Fingerhut (192) separated a water soluble product, lipamin, which they believed to be one of the hormones produced by the ovary. Okintschitz (162) used glycerol mixed with saline to extract the tissue. He found that extracts of mature ovaries of cows and pigs and the extract of ovaries of calves and pigs (follicular apparatus) prevented the rapid atrophy of the uteri of castrates, but that the extract of corpora lutea had no effect. With the exception of the investigations mentioned, all of the investigators prior to 1925 failed to find the hyperemic agent in aqueous extracts. Recently Laqueur, Zondek and Glimm have found that aqueous extracts may be potent. Their papers will be discussed presently.

Beginning with the discovery of Iscovesco (110) that the fat solvents extracted the hormone from the proteins, etc., many variations of the treatment of tissues with organic solvents have been used to obtain the hormone in a purified condition. Iscovesco's procedure was a long drawn out process in which a number of fractions of inactive lipoid were separated by their insolubility in the organic solvents. Fellner's work which was published almost simultaneously with Iscovesco's did not advance the degree of purification of the extracts. Herrmann's (100) paper constitutes the most complete systematic chemical and physiological study of the placental hormone prior to 1920 (Giesy, 84) and for that reason will be discussed in some detail.

The essential steps in Herrmann's procedure are: 1, drying of the hashed tissue in vacuo with subsequent grinding of the dry residue to a fine powder; 2, extraction of the powder in a Soxhlet with ether and alcohol; 3, purification of the lipoid extract by the differential precipitation of the components by various solvents such as acetone, cold ethyl alcohol, and cold methyl alcohol, in which the hormone is soluble; 4, fractional distillation and redistillation of the fractions at 0.06 mm. pressure.

Procedures resembling Herrmann's in many respects have been used by Frank (70) and his collaborators, Doisy (53) and his co-workers,

Dickens, Dodds, and Wright (52), Faust (59), and Hartmann and Isler (96). Faust and Hartmann, apparently following in the footsteps of Herrmann, have improved the procedure and introduced new steps which eliminate some of the inert materials and thereby attain a product whose potency is probably several times greater than that of the product obtained by the original Herrmann procedure. Since most of their assays have been made with immature rabbits, it is impossible for us to estimate their progress in the isolation of the pure hormone.

Ralls, Jordan and Doisy (180) extending earlier observations of Allen and Doisy have greatly simplified the extraction of the hormone from liquor folliculi. The alcoholic extract of the proteins is distilled to dryness, the residue dissolved in dilute alkali and the aqueous solution extracted with ether. The ether is distilled off and the solids fractionated between 70 per cent ethyl alcohol and petroleum ether (B.P. 40°–60°). From 3 to 6 extractions of the alcoholic solution are made with $\frac{1}{4}$ its volume of petroleum ether. Most of the contaminating substances pass into the petroleum ether while the hormone remains in the 70 per cent alcohol. The activity of the solids of the alcoholic solution varies between 25 and 50 rat units per milligram.

Though Laqueur (128), Zondek (217) and Glimm (85) mention the solubility of the hormone in water, we will confine our discussion on this point to Laqueur's papers. Apparently dissatisfied with the existing laborious methods of obtaining the hormone, Laqueur and his co-workers avoided the use of lipoid solvents and tried various methods of extraction based upon the hypothesis that the hormone is water soluble. While their description is meager, it seems that they tried a variety of common protein precipitants, of which colloidal iron gave the most promising results. The liquor folliculi was mixed with 4 volumes of normal saline, and $1\frac{1}{2}$ volumes of 3 per cent colloidal iron were added. The filtrate was as clear as water and contained the hormone. Subsequent purification gave a very pure product, of which less than 0.01 mgm. was sufficient to evoke a positive reaction in a spayed mouse. Dialysis experiments were conducted to determine whether the hormone was present in the colloidal form. Since the hormone passed through parchment and collodion membranes, it was concluded that the hormone exists in "true" solution in water.

An alternative procedure described by Laqueur was the direct extraction of the hormone from liquor folliculi with chloroform. Since the chloroform partially precipitates the proteins one would not expect this process to work smoothly. The chloroform is separated from the

aqueous layer and evaporated to dryness. The residue dissolves readily in water giving a clear solution which possesses almost as great a degree of potency as the original liquor folliculi.

The work of Laqueur and his associates in showing that the hormone may be obtained in a *refined* condition by aqueous extraction must be regarded as one of the real advances in the progress of the isolation of the hormone.

CHEMICAL AND PHYSICAL PROPERTIES. *Solubility.* Iscovesco (110) and Fellner (61) showed that the hormone could readily be extracted from ovaries and placenta with alcohol and other organic solvents. However, their extracts contained much lipin material which notoriously affects the aqueous solubilities of many substances. Herrmann (100) and Fraenkel and Fonda (82) have reported that the hormone is soluble in lipoid solvents but their products were not pure and the question is not settled. Ralls, Jordan and Doisy (180) found that their purified product (0.02 mgm. per R. U.) was soluble in alcohol, ether and acetone but insoluble in water and probably only very slightly soluble in petroleum ether. Laqueur, Hart and deJongh (131) working with their product which had been obtained by aqueous extraction of liquor folliculi found evidence which led them to doubt the solubility of the *pure* hormone in certain lipoid solvents. They have prepared aqueous solutions which upon the evidence of dialysis through a membrane they regard as true solutions. Gustavson (79), Zondek and Brahn (217) and Loewe (138) have reported obtaining aqueous solutions but since they have given no data on the purity of their products, it is difficult to properly evaluate their observations.

Dialysis and adsorption. Since Laqueur found that the hormone in aqueous solution dialyzes through collodion and parchment, he believes that the solution is not colloidal. He noted that the hormone did not appear in the outer fluid in as large concentration as expected and was able to extract it from the membrane. Later it was found that supranorit, talcum and filter paper adsorb the hormone from aqueous solution—in fact three filtrations of a solution through paper were sufficient to remove all of the hormone.

Molecular weight. Little can be said about the molecular weight. Herrmann and Fraenkel determined it but their products were very crude (Hartmann and Isler, 1926) (96). Jordan (180) found an average value of 438 for a product of which at least $\frac{3}{4}$ was not hormone.

Color reactions. Contrary to the earlier reports of Herrmann and Fraenkel, it has been shown (53) that the hormone gives neither the

Liebermann-Burchard nor Salkowski color reactions; this finding has been confirmed by Laqueur (129), Dickens (52), and Gustavson (79).

Laqueur has obtained a negative biuret reaction and by other methods shown the absence of protein in his extracts. The diazo reaction was likewise negative. Doisy and Thayer (unpublished) have tested a purified product with arsenic trichloride which has been used as a test for vitamin A but did not obtain the blue color supposed to be associated with vitamin A.

Composition. The preparations analyzed thus far have been so crude that it is doubtful whether much significance should be attached to the data obtained and only the analyses of the least impure products will be mentioned. In the 25 rat units per milligram product Ralls, Jordan and Doisy found 80.7 per cent C, 10.4 per cent H, 0.93 per cent N and no P. Nitrogen was determined by the micro Dumas method. Laqueur found no nitrogen by a micro Kjeldahl procedure but it is well known that certain forms of nitrogen are not determinable by that procedure. Since it is possible that the nitrogen found by Jordan may have been present in one of the impurities, as at least $\frac{3}{4}$ of the product analyzed was not hormone, the evidence on this point is far from conclusive.

Laqueur found neither hydroxyl nor amino groups by the diazo reaction and Gustavson reported that treatment with PCl_3 did not affect the potency, but Jordan found a loss in activity when the hormone was treated with reagents which react with alcohols and amines, i.e., acetyl or benzoyl chloride or naphthyl isocyanate

Fraenkel reported the analysis of a hydrazone but his data seem questionable in that he did not show that the hormone could be recovered and shown to possess activity. Neither Gustavson nor Jordan obtained any evidence of the presence of a carbonyl group and the latter showed that the residue after treatment with phenylhydrazine possessed the same potency as the original substance.

Many investigators have referred to the presence of double bonds in the hormone but the evidence does not seem conclusive. Hydrogenation (Ralls, Jordan and Doisy) with a very effective platinum oxide catalyst does not impair the activity appreciably We may therefore infer that no double bonds exist in the hormone molecule or that they are of no physiological importance. The loss in activity due to treatment with bromine or potassium permanganate which was reported by Giesy (84) is undoubtedly true, but it may be due to oxidation of some labile group other than a double bond. That some labile group is

TABLE 4

Chemical and physical properties of the hormone

PROPERTY OF REACTION	RESULT	OBSERVER AND REMARKS
1. Solubility		
Water	Insoluble	Herrmann (1915), Allen and Doisy (1923)
	Soluble	Laqueur, Hart and deJongh (1926)
Alcohol	Soluble	Fellner (1913), Iscovesco (1912)
Acetone	Soluble	Fellner (1913)
Ether	Soluble	Herrmann (1915)
Chloroform	Soluble	Herrmann (1915)
Benzene	Soluble	Herrmann, Frank (1915)
Petroleum ether	Soluble	Herrmann
2. Dialysis:		
Collodion	Dialyzes slowly	Laqueur, Hart and deJongh (1926)
Parchment	Is adsorbed by membrane	
3. Adsorption:		
Supra-norit, talcum, filter paper	Adsorbed from aqueous solutions	Laqueur, Hart and deJongh (1926)
4. Molecular weight	438	Ralls, Jordan, Doisy (1926) (May be very inaccurate)
5. Color reactions		
Biuret	Negative	Laqueur, Hart and deJongh (1926)
Liebermann -Burchard Salkowski	Negative	Doisy, Ralls, Allen and Johnston (1924)
Diazo	Negative	Laqueur, Hart and deJongh (1926)
Arsenic trichloride	Brown color	Doisy and Thayer (unpublished)
6. Composition		
C, H, O	Present	Ralls, Jordan and Doisy (1926)
P	Absent	Ralls, Jordan and Doisy
N	Absent	Laqueur, Hart, deJongh and Wijsenbeek (1925)
	0.9 per cent	Ralls, Jordan, Doisy (1926); (N may be due to impurity)
Amino or hydroxyl	Absent	Laqueur, Hart, deJongh and Wijsenbeek—(based on diazo reaction)
	Present	Ralls, Jordan and Doisy (1926) (based on reaction with benzoyl chloride, acetyl chloride and naphthyl isocyanate)

TABLE 4—*Concluded*

PROPERTY OF REACTION	RESULT	OBSERVER AND REMARKS
Carbonyl	Absent	Ralls, Jordan and Doisy (1926)
Double bond	Doubtful	Ralls, Jordan and Doisy (Hydrogenation does not affect potency)
Bromine	Inactivation	Giesy (1920)
$KMnO_4$	Inactivation	Giesy
Saponification with NaOH	No loss of potency	Laqueur, Hart and deJongh (1926)
H_2SO_4 at high temperatures	No loss of potency	Laqueur, Hart and deJongh (1926)
Volatility	Below 200° at 0.06 mm.p.	Herrmann (1915)

present seems to be indicated by some recent work on the effects of exposure to light. Dry residues from lipoid extracts of follicular fluid and placenta and residues dissolved in corn oil lose activity when exposed to ultraviolet rays (Allen and Ellis, 13). Preparations of the hormone containing petroleum ether or certain fluorescent substances lose their potency on exposure to light (Jordan and Doisy, 114). The inactivation is more rapid in ultraviolet or sun light but also occurs in the diffuse light from north windows.

That the hormone is not an ester seems to have been indicated by the work of Doisy, Ralls, Allen and Johnston but established on a much more secure basis by Laqueur, Hart and deJongh who showed that a purified preparation may be boiled with 26 per cent KOH or 26 per cent H_2SO_4 without loss of potency.

Herrmann introduced distillation at 0.06 mm. pressure for the purification of the hormone. This procedure has been used by Ralls, Jordan and Doisy but given up because the product seemed less pure after distillation than before. Hartmann and Isler found that better purification (than Herrmann effected) led to the finding of active hormone in the distillate at a lower temperature (145°).

The physical and chemical data are summarized in table 4 for the convenience of the reader.

PHARMACOLOGICAL PROPERTIES. *Blood pressure.* Although there are numerous reports upon crude extracts, the only data bearing upon the effect of purified preparations upon the blood pressure are those of

Laqueur and his co-workers and Doisy and his collaborators. In 1923 Gruber (Allen and Doisy, 1924) in a single experiment found no change in the blood pressure of an anesthetized dog after the intravenous injection of an active extract prepared from the liquor folliculi. This result has been substantiated by Laqueur (129) who injected 0.1 mgm. (15 mouse units) into a narcotized cat and by Doisy, Ralls and Jordan (54) who found that purified preparations had no effect on blood pressure but that crude extracts had a pronounced depressor effect. Neither the crude nor purified products studied produced any appreciable effect upon the heart rate.

Laqueur also reports that the purified hormone has no effect upon the isolated frog heart, the respiration of narcotized cats or the blood sugar.

Basal metabolism. Though the effects of ovariectomy upon metabolism have been much studied, there does not seem to be complete agreement upon this point. Loewy and Richter (149) found that the basal rate of castrates was raised to normal or even above by the administration of dried ovaries and Zondek and Bernhardt (216) have obtained similar results. More convincing data have been adduced by Laqueur (127) who has studied the metabolism of spayed rats after the injection of Menformon. The results indicate that injection of castrated female rats with this preparation causes a small but definite increase in the production of carbon dioxide and consumption of oxygen.

Toxicity. Repeated injections of purified preparations of the oestrous hormone do not seem to impair the growth of white rats. Furthermore, from 20 to 50 rat units have been injected in a rat in one day without producing any noticeable ill-effects. The kidneys were examined microscopically (unpublished data—Allen and Doisy) but no lesions were detected and no albumin was found in the urine. Laqueur reports that Menformon in doses up to 45 mouse units in both animals and man was non-toxic.

Effect upon testes. Herrmann and Stein (106) reported that the injection of hormone into young male rats and rabbits delayed the attainment of sexual maturity and that prolonged injection of larger quantities of the corpus luteum hormone in adult rats and rabbits led to degenerative changes in the testes. The degenerative changes are similar to those produced by x-rays. Fellner (65) repeating and extending the work of Herrmann and Stein found the same degeneration of the testes produced by injection of the placental or corpus luteum lipoid but also found that the lipoid obtained by the same extraction procedure from the testes introduced parenterally produced the same typical picture

of degeneration of the spermatogenic tissue. Gould and Doisy (86) obtained delayed spermatogenesis after the injection of young rabbits with extracts of liquor folliculi but were disposed in view of the extreme sensitiveness of the testes (Oslund) (163) to consider this as a non-specific effect. Bugbee and Simond (35) report absence of anti-masculine effects from injections of follicular hormone. However, the results of Laqueur (126) with Menformon, which undoubtedly is a preparation more closely approaching purity than any of the others used, indicate that the oestrous hormone may really possess "anti-masculine" properties.

ADDITIONAL EVIDENCE FROM MATURE, OVARIECTOMIZED ANIMALS AS TO THE GENERAL PHYSIOLOGY OF HORMONAL RELATIONSHIPS. *The rat and mouse tests used for identification and standardization.* The advantages in the use of the rat and mouse as test animals have been summarized in table 1. Since identification of small quantities of hormone is desirable especially for chemical fractionation the smallest definite unit should be obtained. This seems to be roughly proportional to body weight. Bugbee and Simond (37) have given a formula for a more exact calculation of the weight factor involved in the rat unit. The mouse unit is considerably less than the rat unit,—probably from $\frac{1}{2}$ to $\frac{1}{8}$ of the latter.

The first precaution must be complete ovariectomy checked by a considerable period of dioestrous smears following operation. The observations of Davenport (51) and Parkes, Fielding and Brambell (176) give warning of the possibility of regeneration of ovarian tissue in mice. However, when the ovarian capsules, uterine tubes and the adjacent half of the uterine cornua are removed with the ovaries in the preparation of test animals, much of the danger of regeneration of ovarian tissues is removed. Daily observations on all test animals during periods in which no injections are made establish a safe control.

In all of the writers' work with the rat and mouse as test animals, tests have been considered positive only when considerable numbers of cornified cells (with absence of leucocytes) constitute the smear, indicating growth to the point of formation of a distinct stratum corneum. This stage should be reached at the proper time interval, namely, the third day or 48 to 60 hours after the first of a series of injections made during the first 24 hours. As a routine, smears are made at approximately 48, 56 and 72 hours or the morning and evening of the third day and the morning of the fourth day of the experiment. Usually the first two are oestrous smears; the last may show the beginning of the leuco-

cytic infiltration characteristic of the metoestrum. The relative number of cornified cells indicates roughly the thickness of the layer developed through hormonal action and also the amount of tissue sloughed.

Recently several investigators, Laqueur (128), Loewe (141) (142) (143), Lipschütz et al. (134), have advocated the use of a pro-oestrous reaction as a positive test. This reduces the minimal effective dose. If this method is rigidly controlled by standardization of technique it may be very useful, but it should be realized that it undoubtedly sacrifices the clear-cut nature of the end-point which has been stressed (table 1) as one of the principal advantages of the vaginal as compared with the uterine reaction as a criterion of activity of ovarian and placental extracts. At least a clear distinction should be made in testing for new sources of the hormone, as for instance extracts of plant tissues. Laqueur insists on the complete disappearance of leucocytes from the smear. In the "cell count" method of Loewe, however, smears containing some leucocytes and relatively few epithelial cells and completely cornified cells might be described as positive results, where in the normal animal this type of smear would be considered as only an early pro-oestrum. It is often difficult to correctly diagnose such a sample. Until the general adoption of a standard unit, results which are reported as positive should distinctly note whether a pro-oestrous or full oestrous condition is used as a criterion of activity and also how the reaction time compares with the normal.

Site of injections. The administration of ovarian preparations has been made by alimentary tract and by intraperitoneal, intravenous and subcutaneous injections. It has been shown that many times the minimal effective subcutaneous dose is ineffective when administered per os (14). Yet the active substance does not seem to be destroyed by certain digestive enzymes (Doisy, Laqueur). Loewe, Lange and Faure (145) have recently reported that 20 units administered per os may cause a vaginal reaction. Evans and Burr (58) have recently shown that smaller doses are effective when injections are made subcutaneously than when they are given intraperitoneally.

Species range of ovarian tests. The species range of activity of this hormone has recently been extended. Besides the mammalian sources already listed in table 2 active material has been obtained from birds (17) (67) and fishes (67). Growth has been reported in the oviducts of pigeons from injections of extracts from mammalian sources (182).

The corpus luteum as a source of this hormone. The emphasis placed

by the earlier investigators upon the corpus luteum as the principal ovarian source of this hormone has already been given extensive consideration. As corpus luteum function is discussed at present there is seldom any distinction made as to the species of animal concerned. The observations already described upon the effects of isolated follicular hormone were greatly accentuated by negative rat tests from similarly prepared extracts of corpora lutea of pigs and cows clipped from ovaries as soon as possible after removal (12) (14). In the collection of these corpora the considerations already outlined as to the normal development of the corpus luteum as a transition structure from the ruptured follicle (page 602) seemed to require a differentiation of material into groups or classes. The first consisted of recent corpora lutea still showing rupture points or containing fluid-filled centers which were probably less than four days old (after ovulation). This group consisted of transition "follicle to corpus luteum" structures. The second group contained solid fully developed corpora lutea which from their size and vascularity could be definitely placed at more than 4 days and which had not yet passed full development. In order to definitely remove the possibility of post-mortem diffusion of tissue fluids (liquor folliculi) both these types were taken only from ovaries which contained no large follicles (14)

In preliminary tests of material obtained from these transitional corpora occasional positive tests were obtained, but as frequently negative tests resulted (14). Only negative tests were obtained from the second group of mature corpora lutea (12) (14). Thinking that perhaps a uterine reaction might be induced without a corresponding vaginal reaction by extracts of corpora lutea, these tests in rats and mice were rigidly checked by histologic studies Contrary to results of earlier investigators these tests indicated relatively little hormone in fully developed corpora from the pig and cow. Therefore it seemed probable to us that the endocrine function of the corpus luteum of the pig and cow as far as genital growth stimulation was concerned had been considerably over-estimated.

Later rat tests of extracts of corpus luteum substance from pigs and cows have been reported as positive (78). The highest yields were apparently obtained from corpora lutea clipped from ovaries which also contained large follicles. The writers consider that more quantitative work is necessary before it is established that fully formed corpora lutea of the pig and cow contain the follicular hormone in appreciable quantities. At present it can be definitely stated that the large follicles

contain much more of this active substance than do the fully developed corpora from pig and cow ovaries. Consequently it would seem that the earlier investigators who used isolated corpora or selected ovaries containing large corpora as the source of extracts were at least discarding the major part of the active substance. It is probable that the solution of this point must await the determination of the quantitative range of this ovarian hormone in the ovaries of these animals at various known stages of the oestrous cycle (6). Until the material extracted is definitely placed according to the time in the cycle, dogmatic statements cannot be justified.

Hormone content of human genital tissues. Extending the use of the rat and mouse tests for activity to the quantitative phase of the problem it has been possible to measure the hormone content of human ovarian tissues removed at operation and placentas obtained at various stages of gestation (167) (16) (99) (53) (52) (60). Human liquor folliculi contains large amounts of this hormone. The contents of cystic follicles from human ovaries have returned positive oestrous reactions. The human corpus luteum of both menstruation and pregnancy also contains this hormone in considerable concentration (as much as 3000 R.U. per kilo) (12) (16), a remarkable contrast to some of the analyses of fully formed pig and cow corpora. Zondek and Aschheim (212) (213) have obtained positive results from implants of human corpora and ovarian interstitial tissue. It seems that at least a quantitative distinction should be made between fully formed ungulate and primate corpora as far as the content of this hormone is concerned.

Tests of human placenta have demonstrated that there are more than 15 rat units of hormone in the placenta at three months while during the fourth month a 100-gram placenta may contain more than 30 rat units (16). The full term placenta averaging between 550 and 650 grams may contain from 200 to 400 rat units. Tests of full term umbilical cords have also given positive results. Tests of extracts of intact human chorionic vesicles during the latter half of the second month, containing apparently normal 12.5 mm. and 20 mm. embryos, have returned positive results.

The species range of this hormone in the placenta and chorionic membranes. The questions why the placenta should contain a substance similar to the essential hormone of the ovaries and to what extent these endocrine functions are related are fascinating problems.

In speaking of the amount of hormone in the placenta little stress has been placed upon the possibility of species differences. In most of

the earlier tests human material was the principal source of supply, but Lane-Claypon and Starling (122) used rabbit tissue. These earlier tests undoubtedly demonstrated the presence of a growth hormone acting upon the uterus and mammary glands

Among monotremes and marsupials where no placental structures are developed, the maintenance of uterine environment during embryonic life apparently needs no placental hormone. It seems quite logical therefore, if this hormone is secreted by the placenta rather than being collected there after secretion by the ovaries, that the placenta has secondarily acquired this function which was at first essentially ovarian. The great diversity among mammals of structural modifications of the tissues making contact between mother and embryo as already outlined on page 603 would lead one to look for wide variation in this endocrine function. It is reasonable to suppose that some of the mammals with simpler types of placenta might reproduce without it. Therefore a survey of hormone content in placentas, or chorionic membranes where definite placentas are not formed, should provide a basis for an understanding of this function

Quantitative tests of the amount of hormone in various tissues of pregnancy from different species of mammals have been made using the rat and mouse tests for activity (16) (8) (174). To date extracts of material from the cow, sheep, horse, pig, dog, cat, rat, and chick have been tested.

Both the whole cotyledonary placentas of the cow during the latter half of gestation and the isolated maternal and embryonic parts extracted separately have returned positive tests (8) (174). There seems to be little quantitative difference in the maternal and embryonic portions of placenta. The hormone is present in considerable amounts in the full term "afterbirth" (embryonic portion alone).

Parkes (174) has reported positive tests from sheep placenta. The full term chorionic membranes of the horse which have the diffuse arrangement of villi contain considerable amounts of active material (8).

Chorions, the mucosa stripped from the pregnant uterus, fetuses, and amniotic fluid from pigs during the latter half of gestation have so far returned only negative results. Tests of zonular placentas of cats and dogs late in pregnancy have also been negative. Tests of small amounts of rat placenta and chorionic membranes of chicks have been negative but larger quantities should be tried before a definite statement is made.

It is highly desirable that additional quantitative tests of placentas and chorionic membranes from various animals at different stages of gestation be made to complete the survey of distribution of this material. Such studies should provide sound evidence for interpretation of the relation between the endocrine functions of ovaries and placenta in mammals. Parkes favors the interpretation that the active substance found in the placenta is collected there after being secreted in the ovaries. If this is so the placenta might serve to protect the embryo from the maternal hormone.

The plant kingdom as a source of this active substance. It has been thought for a long time that feeding animals with certain foods would hasten the appearance of sexual function in immature animals and also in mature animals which were sexually quiescent or "out of season." Heape postulated his "reproductive ferment," gonadin, upon results from the old practice of "flushing sheep." The similarity of the method of extraction of ovarian and placental hormones and that used by Evans and Burr (57) in their work on vitamin E indicates certain similarities. The report of the extraction of insulin-like material from plant sources led Kountz to test extracts of flax and hemp seed by the vaginal smear reaction. In unreported preliminary experiments partial vaginal reactions followed injections. At that time, however, they were considered negative for we were requiring full oestrous reactions in test animals. Recently, using the partial or pro-oestrous reaction, Loewe and his co-workers (147) (140) report positive results from plant tissues. This phase of the problem promises a field of unusual interest.

Results of prolonging the period of injections. In the normal rabbit and ferret which do not ovulate spontaneously a persistence of large follicles may be associated with a continuation of oestrous growth in the genital tract. Sometimes a similar condition is present in rats and mice. It is not uncommon to find extended oestrous growth varying from 2 to 6 or even more days in length. It has been shown that such conditions could be obtained experimentally in ovariectomized rats by the continuation of injections of active extracts (14). It seems probable, however, that more than the minimal dose of extract is required to prevent the occasional appearance of leucocytes in the vaginal contents.

Spontaneous or voluntary activity. Slonaker (194) and Wang (207) have described rhythmic periods of spontaneous activity in rats associated with the oestrous cycle. Rats placed in rotary cages will run many more revolutions during the period of oestrus when large follicles are present in the ovaries than at other times. These writers

and others have shown that removal of the ovaries causes the cessation of these periodic spurts of spontaneous activity. Wang, Richter and Guttmacher (208) report the resumption of periodic activity in ovariectomized animals after ovarian transplantation. Bugbee and Simond (36) record an increase in spontaneous activity of ovariectomized rats following injections of aqueous colloidal solutions of hormone.

Uterine or tubal contraction rate. Following descriptions of characteristic variation in the rate and amplitude of contractions of normal uterine and tubal musculature during the oestrous cycle by Corner (41), Blair (24), Keye (116), and Seckinger (189), and Seckinger and Corner (190), attempts have been made to induce these changes by direct application of extracts to the excised uterus and uterine tube. Seckinger (191) has described a modification of tubal contraction phenomena by corpus luteum extracts which is similar to contractions of normal tubes of the pig during the inter-oestrous periods. Negative results were described following addition of follicular fluid. Frank, Bonham and Gustavson (73), however, have presented evidence that the uterus of the ovariectomized rat in experimental oestrous contracts in the characteristic oestrous manner. Further results have been reported by Brouha and Simonnet (32).

Secretion of the uterus and uterine tubes. The great accumulation of secretion in the uterus of ovariectomized mice and rats in experimental oestrus has already been noted (14). Little attention, however, seems to have been directed to the secretion of the uterine tubes. Courrier (49) has shown that ovariectomy is followed by cessation of secretion of the tubal epithelium and that injections of follicular hormone into the rabbit causes the resumption of secretion by this tissue.

Identification of this hormone in the blood. Recovery of enough of this active substance from the blood to give positive reactions in mice and rats has recently been reported by Loewe (140), Frank and Goldberger (74) (75) (76) (77), Aschheim (18) and Fels (68).

Frank and his collaborators describe positive vaginal tests from blood of pigs in oestrus. Loewe reports highest yields from human blood during the intermenstruum. Frank and Goldberger place the highest yield from human blood just before menstruation. They also find considerable amounts of hormone in menstrual discharge. With the same method they report identification of the hormone in blood from pregnant women as early as the 8th week. Aschheim (18), Fels (68) and Trivino (203) describe positive mouse tests from small amounts of blood serum during the latter part of pregnancy.

Absorption of injected hormone. Little study has so far been directed upon the rate of absorption of oil solutions as compared with aqueous preparations of this hormone. One rather surprising result which emphasizes the reaction time factor is that doses of 10 or 20 rat units injected during the first 24 hours induce oestrous vaginal reactions of but little longer duration than 1 or 2 rat units doses. The optimal experimental condition seems to be the even maintenance of an effective dose rather than its high concentration at any one time.

Ovarian hormone injected into the uterus of ovariectomized rats after ligation of the cervix is readily absorbed with the characteristic vaginal reaction (Allen, 4) (Loewe and Voss, 148).

Excretion of hormone. Positive tests have been reported of material isolated from human urine (144). The yield seemed to vary with the stage of the menstrual cycle (1–2 M.U. per day, using the pro-oestrous cell count method), indicating possibly incapacity of the body to store this hormone. Perhaps this point instead of rate of absorption of injected hormone is the explanation of failure to get proportionately longer oestrous periods from concentrated extracts.

RESULTS FROM INJECTIONS INTO NORMAL ANIMALS (WITH OVARIES INTACT). *During the prenatal period.* An acceleration of growth of the genital tract in the newly born guinea pig from injections of follicular extract into the mother toward the end of gestation has been reported by Courrier (44) (46). This would of course require the passage of the hormone from the maternal circulation through the placenta. In tests of this question in rats and mice negative results have been described (Allen, Frances and Craig, 15), (Parkes and Bellerby, 172). It seems quite possible that a species difference may exist or that toward the end of a long gestation period (more than 60 days in the guinea pig as compared with 20 to 21 in the mouse and rat) the placenta might become more permeable. It is highly desirable, especially after the negative results obtained by Snyder and Hoskins (197) regarding placental permeability to hormones (adrenalin, insulin, pituitrin) in the rat that this question be extensively studied in several species of animals.

During the prepubertal period. Immature females were used as test animals as early as 1912 (Fellner, 60). The aim of these experiments was the premature induction of a sexually mature condition. For the most part in the earlier experiments the extracts used were made from whole ovaries, corpora lutea and placenta. Hyperemia of the external genitalia and whole genital tract and marked growth of the uterus and mammary glands were described (100, and others). This experimen-

tally induced premature genital development when compared with the normal controls was very successful. In some of these experiments immature test animals were spayed (61). In most cases, however, the ovaries were intact in the injected animals. In describing the effects of injections into immature animals several reports included the presence of an unusual number of large follicles in the ovaries. Others, however, noted little effect upon the ovaries. Although the premature development of sexual conditions (at least as far as the first three criteria enumerated above) had been demonstrated by injections of hormone in these early experiments, there still seemed to be a question as to whether these effects, in some of the experiments at least, were obtained secondarily through activation of the follicles of the immature ovaries or primarily through the direct action of the injected hormone upon the genital organs without intermediation of the ovaries.[5]

The use of the immature rat is advantageous for the study of this question. In this immature animal the outer portion of the vagina consists of a solid cord of epithelium without a lumen, consequently it remains closed until the approach of the first oestrous period (Long and Evans, 150). Using fresh liquor folliculi (instead of corpus luteum or whole ovaries) several series of injections were made into 20 to 30 day old rats (11) (80) (214) (67). Rapid proliferation of the vaginal epithelium with formation of the oestrous cornified layer (the fourth criterion) and opening of the vagina resulted. In a 2 or 3 day interval the infantile uterus had become transformed. The almost embryonic type of epithelium had changed to a definite low columnar form, the lumen was distended with secretion and the muscle layers were well differentiated.

These changes in vagina and uterus were effected readily in ovariectomized immature animals. Consequently these results were due to the primary effect of the injected hormone and not to the secondary effects through activation of the ovaries. This point may appear trivial but its importance is emphasized by the recent results obtained by Zondek and Aschheim (215) and by Smith (196) with implants of anterior lobe of the hypophysis. Independently they have demonstrated that several implants into normal immature female rats will hasten sexual development just as does the follicular fluid. They have further shown, however, that such implants have no effect upon the genital development of ovariectomized animals. Consequently the

[5] As has already been pointed out, the phenomena induced in normal animals cannot be considered substitution effects.

anterior lobe must accomplish these effects through the intermediation of the ovaries by inducing development of ovarian follicles which in turn produce the effective hormone.

The mammary glands of the immature animal appear to be influenced less than the vagina and uterus by injections of ovarian hormone (Brouha and Simonnet, 29). Further study of hormonal influence upon these and upon the ovaries is highly desirable.

Normal, mature, non-pregnant animals (with ovaries intact). Injections of this hormone into adult animals in which ovaries are still in place have disclosed some interesting features. Riddle and Tange (182) obtained growth in the oviducts of doves injected during the sexually inactive season.

Courrier (48) and Hisaw (unpublished) have induced oestrous conditions by injections into small mammals (hedgehog and ground squirrel) during hibernation.

Murphey and his collaborators (156) (157) have described some interesting experiments upon sterile cows in which injections of follicular fluid seemed influential in inducing fertile copulation. Concise experimental control is of course difficult in the investigation of this phase of the problem.

Injections into normal rats and mice tend to shorten the degenerative phase of the cycle. Brouha and Simonnet (30) report lengthening of the oestrous phase with, however, a periodic infiltration of leucocytes in such animals.

Asdell and Marshall (21) injected dogs during the period of anoestrum, the sexually inactive period. Marked growth of the vaginal epithelium to stratification and cornification and development and secretion of uterine glands resulted. External signs and mating instincts were not induced in experiments extending over 10 day periods.

Injection effects in pregnant animals. Since the presence of active material has been demonstrated in placental tissues of some of the larger animals, the effects of increasing the amount of the hormone in the circulation during gestation seemed an interesting experiment. In the rat and mouse the vaginal epithelium remains thin (Long and Evans) and consequently the dioestrous smear, composed of a few epithelial cells and varying numbers of leucocytes, continues throughout pregnancy.

Injections of the follicular hormone during pregnancy induced oestrous growth in the vaginal epithelium resulting in positive smear tests (15). In these experiments the same dose was injected at several stages of

gestation. The pregnant rats after the 4th day of gestation tolerated considerable doses of hormone when injected in oil solutions subcutaneously without the interruption of pregnancy. Toward the end of the gestation period, doses which were effective at earlier stages, proved ineffective. Brouha and Simonnet (29) also record negative reactions from moderate doses late in gestation. Although coloring the oil solutions of hormone with fat stains demonstrated the passage of the oil into the embryos, no acceleration of genital growth of embryos was indicated (15).

Using emulsions of extracts of follicular material Smith (195) and Parkes and Bellerby (172) have studied the results of varying the strength of extracts injected. Smith found that during the first 4 or 5 days after insemination and therefore before implantation of the embryo had begun the injection of 3 or more rat units of hormone terminated the pregnancy. During later stages of gestation the interruption of pregnancy was more difficult. These results were interpreted as evidence that the corpus luteum of pregnancy in the rat does not secrete this hormone.

The results of Parkes and Bellerby in mice are similar, in that large doses late in pregnancy resulted in abortion or resorption of embryos. They carried the solution of the problem further by studying the effects of injections into pregnant animals after the ovaries had been removed. After ovariectomy smaller amounts of hormone were effective in inducing the vaginal reaction even late in the gestation period. These results were interpreted as indicating an inhibitory action on the part of the corpora lutea of pregnancy toward the follicular hormone. No acceleration of genital growth was noted in embryos or newborn from injected mothers which would indicate a passage of this hormone through the placenta.

The effects of injections into lactating animals. Experiments to test the effects of injected hormone during lactation have shown that the vaginal reaction can be induced without affecting lactation or influencing the genital organs of nursing young (15) (173). Parkes (173) has shown that several mouse units were required to produce a reaction in lactating animals, but after these animals were ovariectomized, smaller doses of hormone were effective. An inhibitory action by corpora of lactation is considered responsible for this quantitative difference in effects noted.

Sexually senescent animals. Studies upon senescent animals are fragmentary to date. The induction of experimental oestrus in senescent

rats and mice has been reported by Steinach, Heinlein and Weisner (200) and by Laqueur (125). The injections of hormone were followed in the usual reaction time by the typical oestrous smear and in two cases second periods occurred approximately 17 days later without additional injections. In the reports referred to above, control dioestrous conditions were recorded for considerable periods preliminary to the beginning of injections.

EVIDENCE CONCERNING THE MECHANISM OF THE MENSTRUAL CYCLE. Obviously, with the existence of such striking differences between the oestrous cycle of rodents and the menstrual cycle of primates (page 602), results obtained in the lower animals should not be applied directly to interpretations of the menstrual cycle. Consequently injection experiments were begun into the monkey, *Macacus rhesus*, to determine the extent to which this hormone could replace ovarian endocrine function in primates.[6]

The normal conditions have been studied by Heape (97) (98), van Herwerden (204) (205) and recently more extensively by Corner (40). These studies have shown that monkeys have a menstrual cycle very similar to that of woman. In addition the prominent secondary sex characteristic of reddening and swelling of the skin surrounding the external genital organs is present during sexual activity (Collings, 39).

In his study of ovarian and uterine changes Corner recovered two unfertilized ova (one from a tube, the other from a uterus) on the 14th and 17th days respectively after the first appearance of the previous menses. Two other tubal ova have been obtained on the 10th and 14th days of the cycle (Allen, 5). Recovery of these ova made it possible for the first time in primates to correlate the stage of development of recent corpora with the time of ovulation checked by recovery of ova.

Corner's observations definitely established the occurrence of menstruation without ovulation in a considerable number of apparently normal cycles. Corner also contributed an extensive study of cell changes in the vaginal smear, which show that monkeys and man (Lehmann, 132), (King, 117) are alike in this respect.

Later work in monkeys on correlation of previous menstrual histories with the incidence of corpora lutea in the ovaries showed that ovulation occurred in less than 40 per cent of 27 menstrual cycles in 5 monkeys (9).

In the preparation of monkeys for tests of hormonal activity, ovariectomy was performed in the majority of cases during the interval between menses. When the ovaries were removed toward the end of, or immediatly after the follicular phase of the cycle, the appearance of menses

[6] This work in monkeys has been supported largely by a grant from the Committee for Research in Problems of Sex of the National Research Council.

following the operation was considerably hastened (7). These results seemed to indicate that in the normal course of events the development of the corpus luteum or in the absence of ovulation, the atresia of large follicles, might extend the interval between menstrual periods. The fact that profuse menses followed the operative removal of hormonal influence by 4 to 6 days seemed to indicate that menstruation, like the metoestrous leucocytic infiltration of the rodents, was partly due to decrease or absence of hormone stimulation after it had been active through the growth period of the follicles.

After the removal of the ovaries the reddening and swelling of the "sexual skin" faded and decreased, and finally disappeared. In all cases at the end of the second week following ovariectomy the skin of these regions was colorless and tight.

After varying control periods following ovariectomy, injections of extracts of liquor folliculi from pigs and of human placentas were begun (9). A total of 13 series ranging from 9 to 20 days with either 2 or 3 injections daily was completed. The extracts were standardized in rat units and the total doses injected varied from 64 to 230 rat units of hormone. The first sign of activity of injected hormone was the appearance of reddening and swelling of the vulva and surrounding regions. This increased as injections were continued until in several cases maximum conditions had developed. A second criterion of activity was the disappearance of leucocytes from the vaginal contents and the increase in number of epithelial cells. Toward the end of a series of injections partly and completely cornified epithelial elements made up the contents of the smears.

After cessation of injections the color faded and leucocytes reappeared in the vaginal contents. In seven of ten series, bleeding followed the last injection in from 3 to 7 days. The duration of these experimental menses varied from 1 to 6 days. The flow was scant but characteristic. No bleeding occurred during the periods of injections and none followed three series in an animal from which the uterus had been removed.

Histological studies of the genital tissues showed very great growth of the vaginal epithelium, increase in the size of the cervix with growth of the cervical glands, considerable growth in the uterine mucosa and marked growth of the mammary glands. It seemed probable that in some cases maximum conditions had been induced as far as reddening of the "sexual skin" and growth of the vaginal walls and mammary glands were concerned. The endometrium was probably nearer a "late interval" than a "full premenstrual" stage of development. However,

extensive growth of the uterine glands was indicated by many mitoses and the beginning of coiling in some regions. The presence of secretion droplets along the outer border of the epithelium was considered evidence for at least the beginning of secretory processes.

From these experiments the following conclusions seem to be indicated:

1. The injected hormone started the growth processes in the female genital tract and mammary glands and induced the external "sexual skin" characteristics.

2. It induced at least partial growth in the endometrium with a beginning of coiling of the glands and secretion.

3. In case ovulation occurs, the corpus luteum in primates (16) may continue this same anabolic influence for a considerable period.

4. Menstruation seems to be due to a decrease in amount or temporary absence of this hormone after it has been acting for a time. Menstruation is therefore probably analogous to the leucocytic infiltration of the metoestrum in rodents.

5. Apparently a specific hormone from the corpus luteum, different from that of the follicle, does not seem to be a necessary causal factor in the essential mechanism of the menstrual cycle. However, full premenstrual growth in primates may require continued action of this (or perhaps another) hormone.

6. Lipoid extracts of human placenta are as effective as extracts of follicular fluid in inducing these effects. The continuous availability of this substance in the human placenta throughout gestation might partly account for the absence of menstruation during pregnancy.

7. The essential ovarian endocrine mechanism of the non-pregnant menstrual cycle seems to be little different qualitatively from that of lower mammals. Quantitatively and in reaction time there are differences. Perhaps full premenstrual growth of the endometrium with growth and secretion continued after ovulation can be explained in primates upon these grounds.

HORMONES FROM THE OVARIES AND PLACENTA OTHER THAN THE "GROWTH, SECRETION, MATING-REACTION INDUCING SUBSTANCE." Marshall has postulated at least three hormones from the ovaries; one from interstitial tissue determining the development of the genital organs and secondary sex characteristics before puberty, one from the follicle causing oestrus and one from the corpus luteum responsible for uterine and mammary growth. It has been demonstrated that an active substance which to a certain extent fulfills these requirements can be ex-

tracted from follicular contents, from placentas and from corpora lutea at least of primates.

The recent x-ray results of Parkes already referred to seem to indicate that after destruction of follicles the remaining ovarian tissue may retain its function of production of hormone. If this new proliferation of ovarian tissue is identical to "interstitial tissue" of normal ovaries Parkes' work is strong substantiation of hormonal function of this tissue.

Whether this substance produced by the different ovarian tissues varies in chemical constitution at different phases of sexual life is still an open question which must await further chemical advances for solution.

Other hormones from the ovaries have been described. Certain normal phenomena of reproduction which might depend upon hormonal control have not yet been obtained by substitution experiments with extracts. The pseudo-pregnant condition of the uterus is an instance and the "deciduomata" reaction of Leob (135) has not yet been successfully obtained in injected ovariectomized animals.

From early hypotheses concerning the corpus luteum the question of inhibition of growth of ovarian follicles has been prominent. Excision experiments of Loeb (136), of Papanicolaou (164), and others resulted in more rapid growth of follicles. Whether this was due to removal of an inhibitory corpus luteum hormone or merely to intra-ovarian nutritional balance has been discussed extensively by Hammond (91) and others. Considerable evidence on this phase of the question has already been advanced.

Concerning an inhibitory corpus luteum hormone. Pearl and Surface (177) reported inhibition of ovulation in the fowl by intraperitoneal administration of a fat free preparation of corpus luteum substance. Loeb (136) and Corner and Hurni (43) reported failure of corpus luteum extracts to inhibit ovulation in rodents.

Haberlandt (88) (89), Knaus (119) and Kennedy (115) describe temporary sterility of females injected with corpus luteum extracts.

Papanicolaou (165) (166) reports the inhibition of oestrus determined by the smear method in the guinea pig over extended periods of time by injections of the lipoid fraction of corpus luteum extracts. Negative results were obtained in control tests of follicular fluid, placenta, thyroid, thymus and other tissues. Papanicolaou considers this the "proper luteal hormone" and states that it may operate to inhibit follicular growth at the same time that the corpus is continuing the secretion of the genital growth hormone of the follicle.

The problem has been approached by Durant (55) from the point of view of the periodic spontaneous activity associated with the oestrous cycle in rats. He reported that subcutaneous injections of extracts of corpora lutea continued for thirty-seven days in normal rats failed to suppress these periods of spontaneous activity.

The work of Parkes and Bellerby (172) in which they obtained vaginal reactions in pregnant mice with smaller doses of follicular fluid after ovariectomy has already been cited. They attributed these results to the removal of corpus luteum inhibition.

Further work on this problem may definitely establish this as a specific effect of corpus luteum extracts upon follicular growth and thus upon the appearance of oestrus. As far as the writers are able to find, no successful attempts to inhibit the action of injected follicular hormone by the injection of corpus luteum extracts have been reported.

Papanicolaou (165) considers that there is a third ovarian hormone present in the water soluble fraction of residues of alcoholic extracts of follicular fluid. The action of this extract is to further follicular development and slightly shorten the interval between oestrus periods. Unlike other ovarian hormones, this is active when administered by mouth.

Hisaw (107) describes the dissolution of the symphysis pubis in the normal and castrated male pocket gopher by injections of saline extracts of ovaries. He also describes relaxation of pubic ligaments in virgin guinea pigs injected during the early postoestrum with blood serum from pregnant rabbits and extracts of rabbit placenta (108). Similar results can be obtained in ovariectomized guinea pigs in experimental oestrus induced by follicular hormone. Serum from the pregnant cat, dog or rat was ineffective. Hisaw's work is opening up an especially interesting phase of hormonal control.

NAMES PROPOSED BY DIFFERENT INVESTIGATORS. Besides trade names used for commercial ovarian products, different investigators have already proposed several for this hormone. Courrier prefers "folliculine," indicative of the principal ovarian source. Parkes and Bellerby suggest "oestrin" to indicate its action in producing oestrus or "heat." Laqueur calls his highly purified produce "menformon" and stipulates a definite degree of purification and a unit requirement of 10 mouse units per 1 cc. Until an explanation of the high yield of active substance from the placenta is forthcoming, the name "folliculine" seems misleading. "Oestrin" from its derivation seems to emphasize the "sexual heat" or "mating instincts" of the lower mammals. Further advances may possibly suggest a more appropriate name.

CLINICAL APPLICATIONS OF THESE RESULTS. *Ovarian preparations for clinical use.* Most commercial ovarian extracts at present on the market and in wide clinical use have not been subjected to standardization by biological testing. Geist and Harris (83) using the uterine reaction in the rabbit, reported negative results from extensive tests. That attempted substitution therapy by administration of ovarian preparations by mouth is ineffective has been demonstrated (14). Lipoid extracts of ovarian tablets have been negative when injected subcutaneously. Subcutaneous injections made during 1924 and 1925 of commercial ovarian extracts, even when the contents of as many as five 1 cc. ampules were injected into a single rat in 24 hours, have returned negative vaginal reactions (14). It seems probable that large amounts of active material had previously been discarded in chemical fractionation of commercial preparations.

For more than a year, however, active preparations have been available for clinical use in ovarian disorders. These preparations are standardized in rat units. The writers have checked this standardization of products from several firms and found them to contain the labelled strength. At least obstetricians and gynecologists using these preparations will have the satisfaction of knowing that they are working with biologically standardized material.

Encouraging results have already been reported from therapeutic use of active preparations in cases of ovarian disorders by Adler (1), Seitz, Wintz and Fingerhut (192), Herrmann (103), Guggisberg (87), Zondek (211), Pratt (178) (179), Laqueur (123), Novak (161) and Brouha and Simonnet (31). Work on this phase of the problem promises worthwhile results.

GENERAL SUMMARY

There may be several hormones produced by the ovaries and placenta. Partial chemical isolation of at least one substance from these sources has been accomplished. No differentiation has yet been made in material from ovaries and placenta. Characteristic replacement effects have been demonstrated by injections of this active substance.

The essential steps in chemical preparation and the properties of the most highly purified products are reviewed.

The reactions induced in ovariectomized animals are 1, hyperemia and growth in all parts of the reproductive tract and in the mammary glands; 2, secretion by uterine glands and the epithelium of the uterine tubes; 3, typical contraction rhythm of uterine and tubal musculature;

4, congestion, swelling and other external signs of oestrus; 5, typical female mating reactions and 6, scanty menstruation (in monkeys) after injections were stopped.

These results provide experimental data for the comparison of the essential mechanism of the oestrus cycle in lower mammals and the menstrual cycle in primates. They should also advance the therapeutic use of ovarian extracts in the clinical treatment of ovarian disorders.

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